THE
JOURNAL OF
COMPARATIVE PATHOLOGY
AND
THERAPEUTICS.

Vol. XLIX.—No. 3. SEPTEMBER 30th, 1936. PRICE 4s.

THE LIFE-HISTORY OF *PSOROPTES COMMUNIS* VAR. *OVIS* WITH PARTICULAR REFERENCE TO LATENT OR SUPPRESSED SCAB.

By W. DOWNING, F.R.C.V.S.

(*Continued from page* 180.)

III.—THE CLINICAL ASPECT OF SHEEP SCAB.

In its clinical aspect, sheep scab might be divided into three phases:—

(1) Active sheep scab in the winter.
(2) Active sheep scab in the summer.
(3) Latent or suppressed sheep scab.

(1) *Active Sheep Scab in the Winter.*

Sheep scab during the winter months, especially in frosty weather, progresses very rapidly over the animal's body and, as the writer has seen, extends in very advanced cases down the legs to the coronets and up the neck, on to the head, face and lips. Sheep artificially infected in the winter may, in six weeks to two months, be covered with crusts or show denudation over at least three-quarters of the body surface. For instance, a batch of nine sheep were artificially infected on September 4th, 1934, with a small number of ovigerous females and pairs *in copula*. By October 17th the disease was sufficiently advanced (at least a half to three-quarters of the body infested) for the sheep to be used for a dipping trial. On the second occasion nine sheep were infected each with five to six ovigerous females and three to four coupled pairs on October 11th. On October 16th two sheep showed no active lesions and were reinfected. On all the others there were small but active lesions. On December 10th the nine sheep were sufficiently advanced cases to be used in a dipping trial and on two of the sheep the scab had extended on to the head.

The sheep referred to above were all of the Hampshire Down sheep, which breed tolerates the close confinement which such

experiments involve better than any other breed. A limited number of observations on Welsh Mountain and Border Leicester sheep suggested that these breeds, which are of an open-woolled type, showed a reduced tendency to exhibit the dense crust formation in comparison with the close-woolled sheep. Also, the symptoms of irritation, such as rubbing and scratching, were not so vigorous.

The experience with the Welsh Mountain sheep was of interest in view of the popular belief that sheep scab in this breed is frequently confined to the tail. On October 19th, 1934, five long-tailed Welsh sheep were penned with some infected Hampshires. No attempts were made to infect the sheep artificially. On November 20th two of the five showed active lesions on the tail but none on the body. One sheep had active lesions on the body, but not on the tail. On the body of the fourth were two lesions which had dried up and on which no acari could be found. The fifth was negative. On December 3rd the lesions on the first two sheep were still confined to the tails. The third and fourth had lesions on the bodies and the tails, whilst the fifth was still clear. On February 5th, 1935, the scab had advanced to such an extent over the bodies and tails that it was necessary to dip the sheep. This double-dipping was successful in eradicating the scab. That the sheep had not contracted scab until November 20th was probably due to the fact that on arrival at the Research Station the Welsh sheep, not accustomed to feeding from troughs or hay-nets, kept themselves aloof from the Hampshires for at least two weeks, during which time they made tentative visits to the feeding troughs and nets after the Hampshires had fed and moved away. Later they mingled freely with the Hampshires and quickly contracted scab.

In winter no difficulty has been experienced in finding acari around the moist edges of the lesions. As has been mentioned previously, on nine sheep infected on October 11th it was possible to observe lesions and acari five days later. In fact, lesions are produced as soon as the acari commence to feed.

As far as the writer's observations suggest, there does not appear to be any variation in the susceptibility to infection due to age. No difficulty has been experienced in infecting clean sheep up to the age of four or five years. Young lambs are equally susceptible. On two occasions lambs were borne by ewes infected with scab. On one lamb acari and lesions were present four days after birth, and on the other a week after birth.

(2) *Active Scab in the Summer.*

During the summer of 1934 three experimental cases of active scab were kept under observation; the sheep (Nos. 10, 47 and 250) showed the clinical symptoms of rubbing and scratching. It is of interest to note that these three sheep were infected in the late

spring and that it was not until the end of the summer or the following autumn or winter that the infestation had progressed sufficiently to allow the sheep to be used in a dipping trial. When this is compared with the results obtained in connection with artificial infection in the winter, where a period of from six weeks to two months was sufficient to allow the disease to reach the same stage, it can be realised how much more slowly the disease progresses in the summer. This is due, undoubtedly, to the slower rate of egg-laying, as indicated in Table IX (page 178).

The histories of the three sheep are given below :—

Sheep No. 250.

This sheep was infected on 24.4.34 with 15 ovigerous females and five coupled pairs.

27.4.34. A small lesion had formed at the point of infestation on the withers and acari were present.

7.6.34. Three coupled pairs of mites from a sheep on which the lesions were spontaneously becoming inactive, were put in a cell on No. 250. The cell became partially detached the next day and the acari escaped. The lesion on the withers was still present, but acari were not numerous.

9.7.34. An active lesion was present on the side of the left shoulder, but not more than six to nine inches in diameter. Five ovigerous females and numerous immature stages were found. The females were transferred to a cell on the side of the sheep. The females laid 16 eggs in six days, an average of 0·5 eggs per day, as opposed to an average of 5·59 eggs in the depth of winter, Table VIII(*c*) (page 177). The eggs hatched to larvæ in three days. The females then escaped from the cell.

30.7.34. The lesion observed on 9.7.34 was active, but had increased very little in size. All stages of acari were observed.

29.8.34. Active lesions were found on the left shoulder and on the side at the site of the cells of 9.7.34 and all stages of acari were present, but not in large numbers.

4.9.34. The scab was sufficiently active to provide sufficient ovigerous females and coupled pairs to infect nine sheep.

17.12.34. The sheep was used in a dipping trial, being a very advanced case of scab.

Sheep No. 10.

25.5.34. Infected with two coupled pairs.

7.6.34. As the scab was developing very slowly, the sheep was reinfected with six ovigerous females and three coupled pairs.

21.7.34. Two hundred acari of all stages were transferred from another sheep, No. 47.

30.7.34. Active scab and numerous acari were present along the back. All stages of acari, including eggs, were found and also colonies in the inguinal folds.

31.8.34. The scab was active, covering the whole body and half-way up the neck.

This sheep would appear to have progressed almost as rapidly as those infected in the winter, but there is a vast difference in the amount of material transferred; over 200 acari of all stages,

compared with five to ten ovigerous females and three to four coupled pairs, which is the number usually transferred in the winter.

Sheep No. 47.

24.4.34. This sheep was infected with 15 ovigerous females.

27.4.34. The females were still present, eggs had been laid and a lesion formed.

7.6.34. Active scab and all stages of acari were found on the withers. Six ovigerous females were transferred to sheep No. 10.

20.6.34. The scab was still active and acari were numerous around the withers.

21.6.34. Two hundred acari of all stages were transferred to No. 10.

7.8.34. The scab was still active and had spread the whole way along the back and partly down the sides.

31.8.34. During the month of August the scab had progressed more rapidly and had affected the whole of the body, the hind legs down to the coronets and the upper surface of the neck.

In the above notes the lesions are referred to as active, when there was a moist area surrounding the crusts, such as is found in the winter.

(3) *Latent or Suppressed Scab.*

As mentioned previously (page 165), the writer and his colleagues at the Cooper Field Research Station have, for a number of years, observed that sheep which have been heavily infested with scab during the winter months, on the approach of warmer weather in spring and early summer, undergo, although untreated, a progressive recovery, so that by midsummer the scab has disappeared and the symptoms of the disease abated. In the autumn the same sheep have relapsed into cases of active scab.

Below are given the details of eleven cases of sheep which were under observation during the summers of 1933, 1934 and 1935. The purpose of the observations was to ascertain whether the scab mite underwent, during the summer months, some hitherto undescribed phase in its life-history, comparable with the hypopial nymphal phase which occurs in many *Tyroglyphid* mites under certain conditions, or with that remarkable Sarcoptid mite infesting pigeons, *Falculifer rostratus*, which lives in the feather quills. On approach of the moulting period, the nymphs of the latter migrate *via* the feather papillæ into the body of the pigeon and pass through a kind of hypopial nymphal phase in the connective tissues surrounding the large blood vessels of the heart, on the pericardium and in the subcutaneous tissues of the neck and breast. When the moulted bird has acquired its new plumage, the temporary quiescent nymphs resume their activity and return to the feather quills. My observations lead me to believe that there is no manifestation of a hypopial phase in the life-history of the *Psoroptes communis* var. *ovis*.

Throughout the summer months acari could be found at all times on eight out of the eleven sheep. In the cases of the remaining three sheep, the infection died out completely and no recrudescence occurred in the autumn. Five of the sheep on which acari were found in the summer became cases of active scab in the winter. The ninth sheep died and two were dipped before the end of the latent period.

At the commencement of this latent or suppressed phase of scab the sheep may harbour numerous acari and their bodies may be extensively denuded of wool and covered with crusts. This latent phase of the disease sometimes commences as early as the end of February or the beginning of March, or, on the other hand, as late as the end of April. It appears to be dependent upon climatic conditions; a cold spring with late frosts usually delays its onset.

The first indication of the onset of latency is the difficulty of finding acari on the sheep, although the latter may still be showing symptoms of pruritus. New wool begins to grow on the denuded areas and as the summer progresses the growth of wool lifts the crusts from the skin. The latter, instead of being wrinkled and indurated as is the case at the onset of latency, slowly recovers and regains its normal condition. Towards the end of August or in September in cases of relapse the typical symptoms of active scab reappear.

There are, however, variations in this history. In the records below, No. 3 and No. 409 were under observation during two summers. During the second summer it was noticeable that there was a less tendency for the crusts to lift off the skin, with the result that the latter never completely regained its normal condition. Also, when the scab became active again it did not spread nearly so rapidly as on a recently-infected sheep.

The fully-developed active lesion consists of dry crusts around which are moist crusts and beyond these a moist zone upon which the acari are found. In a latent lesion small dry crusted areas are to be found on various parts of the body. These areas are not usually more than three to six inches in diameter and in amongst the dry crusts a few ovigerous females may be found, isolated in some cases, in others in association with a few immature acari. Occasionally the acari are to be found under the moist, thin crusts, or, alternatively, they may occur on a patch of scurfy skin forming a lesion of not more than a quarter of an inch or so in diameter. Another type of latent infection may be observed in which isolated lesions do not appear, but acari may be found in the wrinkles of the thickened and corrugated skin.

Acari may also be found in the infra-orbital fossæ and inguinal folds and in such cases are concealed under the inspissated secretions and crusts of the skin glands. Such sheep may exhibit no other lesions on the body.

(*a*) *Records of Cases.*

Sheep No. 409.

This sheep had active scab during December 1932 and January 1933, during which latter month the sheep was used for the rearing of acari in cells. During February the lesions had extended over the body, but not the limbs, head or neck.

30.3.33. Latency had definitely commenced. Only five ovigerous females, two coupled pairs, two pubescent females and three nymphs could be found after a prolonged search of the body. The crusts appeared to be lifting from off the skin, due to the commencement of wool growth.

17.5.33. Only one ovigerous female was found in the crusts on the body. Colonies of acari, about 20 in number and of all stages except eggs, were found in each inguinal fold. The wool had grown to about a quarter of an inch in length, lifting the crusts.

24.5.33. The colonies were still present in the inguinal folds.

20.7.33. In the right inguinal fold, one ovigerous female and a few immature stages were found amongst the crusts. One ovigerous female was on a latent lesion at the base of the scrotum. The crusts of the inguinal fold were not disturbed, so it was not known if eggs were present or not. The sheep was showing no active symptoms of scab.

4.9.33. One ovigerous female was found in the right inguinal fold. An active lesion had developed on the withers. Ovigerous females, nymphs and larvæ were present. The wool had grown on the previously affected parts of the body, to its normal length and the skin was in good condition. The sheep was showing clinical symptoms of active scab.

8.11.33. Active colonies were present in both inguinal folds. The lesion on the withers was still active and another had developed on the inside of the left thigh, three inches above the base of the scrotum. Ovigerous females, coupled pairs, nymphs, larvæ and eggs were found.

The scab on the sheep remained in the active condition during the winter, although it did not develop very rapidly.

10.4.34. Acari of all stages were found in the inguinal folds, as in 1933. The rest of the body was not examined on this date.

11.5.34. A moist lesion two inches in diameter was found behind the right shoulder. Three ovigerous females, two males, one nymph and one larva were present. One female, one nymph and one larva were mounted and examined. They were normal in appearance. One ovigerous female was found in the right inguinal fold. The skin of the sheep was still crusted and wrinkled.

14.6.34. Sixteen ovigerous females (one dead), one male, one larva and one egg were found in the folds of the wrinkles on the right side of the back and withers. The bases of the folds were moist. One ovigerous female was present in the right inguinal fold. The crusts were lifting on the other parts of the body and the sheep showed few symptoms of irritation.

8.8.34. One moulting nymph was found on a latent lesion in front of the right shoulder. A colony of acari was present in the folds and scurf of the right shoulder. The lesion was a quarter to a half inch in diameter. Four ovigerous females, one male, one pubescent female and one egg were transferred to a cell on Sheep No. 401. The skin was still wrinkled and very scurfy, especially on the withers and back. The wrinkles were, however, less

pronounced. The sheep was showing no symptoms of irritation and the labial reflex was a slight licking of the lips. The inguinal folds were free from acari.

14.8.34. Three ovigerous females, one moulting nymph and one larva were found on the lesion behind the right shoulder.

4.9.34. Two small lesions were present at the base of the neck. One ovigerous female and one egg were found on one lesion and one ovigerous female, one pubescent female, one larva and one egg on the other. The colony of acari were still present in the area around the withers. The lesion had increased in size as though the acari had been feeding. The sheep was scratching and rubbing.

16.10.34. Numerous acari in all stages, especially ovigerous females, were present in the folds at the base of the neck, behind the withers and in the inguinal folds. The lesions were much larger than on 4.9.34 and were typically active. The skin was still very scurfy and crusted.

23.10.34. The sheep was double-dipped to cure the scab. The disease had run a more chronic course than in the winter of 1932 and 1933.

Sheep No. 3.

This sheep was infected with scab in October 1932. During the winter 1932-33 the infestation had spread over the whole body surface.

22.3.33. The sheep's body was covered with crusts, but few acari could be found. A colony of acari was present in the left inguinal fold. The sheep was showing clinical signs of irritation.

27.4.33. Acari were only found in the inguinal folds. Some specimens were removed for microscopic examination. These proved normal morphologically. The wool had commenced to grow.

24.5.33. Acari were found only in the inguinal folds.

20.6.33. One ovigerous female was found in the right inguinal fold and one ovigerous female, one male and one larva in the left fold. The sheep was showing very slight symptoms of irritation by occasionally scratching with a hind foot.

23.6.33. No acari were observed in the inguinal folds.

21.7.33. A small moist lesion, larger than a latent lesion was found on the perineum. Two ovigerous females and one moulting pubescent female were found. A small active lesion and one ovigerous female were found on the back. The wool had grown and the crusts were lifted well up in the wool.

23.8.33. An active lesion was present on the posterior part of the perineum and buttocks. A few acari of all stages were found. The sheep was only showing occasional symptoms of irritation.

4.9.33. The lesions of 23.8.33 were still present and in addition one had developed on the base of the scrotum. Acari, chiefly ovigerous females and nymphs were found. The sheep was still showing only occasional symptoms of irritation.

26.9.33-24.10.33. The sheep was used for the observation, in a cell, of an ovigerous female from a latent lesion. As the female laid no eggs and finally died, no fresh infestation of the sheep was made.

3.11.33. The active lesions on the perineal region had developed very slowly. A few acari of all stages were found. A number (approximately 20) of all stages were transferred to the withers from another infected sheep.

8.11.33. The infestation on the withers had been successful ; the acari had fed, crusts had formed, and the females laid eggs.

26.3.34. During the winter, the infestation had spread over the whole body of the sheep, although it had progressed slowly. On this date difficulty was experienced in finding acari on the body, which was very crusted. Two ovigerous females were found in the left inguinal fold.

10.4.34. One ovigerous female, two nymphs and two larvæ were found in the wrinkles on the right side of the body behind the withers. The female was examined microscopically and found to be normal morphologically and to contain an egg.

11.5.34. Two latent lesions were found in the middle of the right side. One solitary ovigerous female was found on each lesion. The inguinal folds were much crusted but no acari were found. The wool had commenced to grow, but the wrinkles in the skin were still very pronounced and the skin itself dry and crusted.

30.7.34. The latent lesions were still present on the right side of the body. One ovigerous female was found on one lesion and one ovigerous female, one nymph and one larva on the other. A latent lesion with one moulting pubescent female and one nymph was found on the right side of the base of the scrotum. The wool had completely grown but the skin was still wrinkled and scurfy. The sheep was not showing any symptoms of irritation.

29.8.34. The sheep showed a slight labial reflex when the back was handled. Two latent lesions but no acari were found. A colony of acari was found in the left inguinal fold, including immature stages, but only one ovigerous female and no males.

4.9.34. Two latent lesions were found on the right side of the body, with one ovigerous female on each lesion. These did not appear to be very active. No other stages were found despite a thorough search. The colony of ascari was still present in the left inguinal fold. This contained all stages of acari, including eggs.

16.10.34. A small active lesion was found on the left side of the body, on which were a number of immature stages. On the right side behind the withers, four ovigerous females, a coupled pair, immature stages and eggs were found. The colony was still present in the left inguinal fold. The skin was still wrinkled and scurfy and the sheep was showing clinical signs of scab.

23.10.34. The sheep was double-dipped. The scab had run a more chronic course than in 1933.

Sheep No. 36.

This sheep had had active scab during the winter 1932-33.

22.3.33. Typical lesions of latency were observed. The body was covered with large crusts, which, on removal, left raw sores. Only solitary ovigerous females could be found among the crusts. In no case were the moist areas more than a quarter to half an inch in diameter. The sheep was still showing symptoms of irritation.

1.6.33. Solitary ovigerous females on latent lesions were found on the perineum. Acari were also present in the infra-orbital fossæ.

29.6.33. Three ovigerous females were taken from the inguinal fold of sheep No. 409 and put in a cell on the back of this sheep, but owing to the wrinkled condition of the skin the cell became loose and the acari escaped.

24.7.33. No acari could be found on any part of the sheep on this date. This sheep was isolated with No. 24.

23.8.33. The sheep was still negative and no acari were found. The wool had grown and the skin improved in condition.

6.9.33. Neither acari nor lesions could be found after a very critical search. The sheep was not showing any of the symptoms of sheep scab.

27.9.33. Neither acari nor lesions were found. The sheep had regained its normal condition except at the site of an old abscess on the shoulders, which was scurfy.

8.10.33. The sheep was still free from scab and was destroyed.

Sheep No. 24.

This sheep had had active scab in the early spring of 1933. The scab had completely covered the body.

17.4.33. Only a few acari could be found on the body, among the crusts, which were very extensive. The sheep was showing symptoms of irritation.

21.6.33. Only one ovigerous female was found on the perineal region.

24.7.33. Neither acari nor lesions could be found on the sheep after an extensive search. The sheep was isolated with No. 36.

23.8.33. No acari were found.

6.9.33. The sheep was still free from scab. The wool had grown and the skin was normal.

27.9.33. The sheep was still free from scab.

9.11.33. The sheep was still free from scab.

23.1.34. The sheep was still free from scab. Acari in cells were put on the sheep on this date.

Lamb No. 2.

10.4.33. This lamb was used for rearing colonies of acari in cells but owing to the escape of acari it had become heavily infected. The lesions had extended to the head, face, tail and legs, as far down as the coronets. All stages of acari, including eggs, were found. Coupled pairs were more numerous than usual. The skin was very crusted and leathery but the moist lesions few and small in size. The body was quite bare of wool.

17.4.33. Numerous acari could still be found, especially coupled pairs. Very few immature stages and no eggs were observed. The acari were found on those parts of the body, such as the face, neck, scrotum and, to a lesser extent, the tail, where there was a covering of long wool or hair. The wool on the body had just commenced to grow and was of a dark brown colour, unusual in the Hampshire breed of sheep.

26.4.33. Many coupled pairs and single males were still present on the lamb. The ovigerous females and immature stages were few. No eggs were found. The acari were found chiefly on the head, neck and scrotum.

4.5.33. Colonies of acari were found on the face and in the inguinal folds. No acari were found in the infra-orbital fossæ.

15.5.33. Eight ovigerous females and one pubescent female were found on the face after an extensive search. On microscopic examination, these all appeared normal morphologically.

24.5.33. A few acari, mostly ovigerous females, were found on latent lesions on the perineum. The wool had begun to grow, lifting the larger crusts from the skin. The lamb was showing only occasional symptoms of irritation.

28.6.33. Latent lesions were present on the perineum at the base of the scrotum on which were found one coupled pair and one larva. Although the wool had grown, the skin was still scurfy.

12.7.33. The lesions were still present on the base of the scrotum but had not increased in size. One ovigerous female and three coupled pairs were found. Two pairs were transferred to a cell on the back of the lamb, but they escaped the next day.

18.7.33. Small lesions, one inch in diameter, were present on the base of the scrotum and on the back. Three ovigerous females were found on the scrotal lesion and two on that on the back.

24.7.33. The lesions were the same as on 18.7.33. Ovigerous females were found. Thirty acari of all stages, except eggs, were transferred to the withers from another sheep which was ill and likely to die.

31.7.33. A moist lesion six inches in diameter was found on the left side of the withers near where the acari were placed on 24.7.33. All stages, except eggs and coupled pairs, were found. The acari had fed and vesicles had formed. The lesion on the perineum with a few acari was still present. The wool had grown and the skin, although scurfy, had practically regained its normal condition. The lamb was slightly inclined to rub.

17.8.33. All stages of acari, including eggs, were found on the withers, although the lesion had not increased to any great extent. There was a small lesion on the perineum on which one ovigerous female and three immature acari were found.

4.9.33. An active lesion was found on the withers, on which were all stages of acari, including eggs. The lesion on the perineum had not increased in size. Three ovigerous females were present on it.

27.9.33. The active lesion on the withers (4.9.33) was still present and all stages of acari, including pairs, were found. The lamb was showing all the clinical symptoms of scab.

24.10.33. The lesion on the back had not increased in size and the acari were few in number. In front of the prepuce a very active lesion had developed on which all stages of acari were found. Males and coupled acari were scarce.

7.11.33. An active lesion was found on the spine on which were ovigerous females, a male and eggs. Single ovigerous females were present on moist lesions on the withers. The lesion around the prepuce had increased and was very active ; numerous acari in all stages were found.

Sheep No. 394.

25.1.34. This sheep had been dipped in a standard carbolic fluid sheep dip on 21.12.33 and infected on 12.1.34. On 25.1.34 an active lesion with acari had developed.

30.1.34. The scab was developing rapidly.

10.4.34. The scab had not completely covered the whole body surface, although a major portion was covered with crusts and denuded of wool. Few acari could be found amongst the crusts.

30.7.34. A latent lesion with one ovigerous female was found on the back. The sheep was showing no symptoms of irritation. The wool had grown and the majority of the crusts had been lifted from the skin.

14.8.34. Latent lesions were found on the left side of the body, on which were ovigerous females. No other stages were observed.

21.8.34. A latent lesion was found on the left side behind the withers on which were two ovigerous females, one nymph and one larva. The

nymph and larva were examined microscopically. The nymph was in the process of moulting to a male and the larva was normal.

29.8.34. The lesion on the left side had become active. Acari of all stages were found, including three coupled pairs. The wool had completely grown and the skin regained its normal condition. Symptoms of irritation, such as rubbing and scratching, were noted.

5.9.34. Active lesions were present on the left side of the withers in the region of the mid-spine, on the scrotum and behind the sheath. A few acari of all stages, including eggs but no pairs, were found on the lesions. Vesicles were present where the acari had fed. The sheep was showing clinical symptoms of active scab.

16.10.34. The active lesions were present as on 5.9.34. A number of acari in all stages, except coupled pairs, were observed. The disease did not appear to be spreading very rapidly. The skin was not covered with crusts, except on the lesions.

23.10.34. Sheep double-dipped to cure the scab.

Sheep No. 340.

This sheep was infected by transference of acari on 4.4.35, 29.4.35 and 15.5.35. On the first two occasions the infection progressed very slowly. The third transference was followed by a week of very cold weather during which one night of 6° of frost and two nights of 2° of frost were experienced.

24.6.35. The scab was still active and by this date had covered the whole body surface. Numerous acari, including pairs, were found on various parts of the body.

1.7.35. Numerous acari could still be found on moist areas on various parts of the body.

16.7.35. Active lesions and acari were found on the face and head and acari were also present in the infra-orbital fossæ, on the chest and in the inguinal folds. The skin was covered with crusts and the sheep was showing symptoms of irritation.

22.7.35. Active lesions and acari were still present on the face and chest and acari were also present in the infra-orbital fossæ and inguinal folds. The sheep was still showing symptoms of irritation.

1.8.35. The lesions on the face appeared to have dried up and no acari were found. A few solitary acari were found on the body amongst the crusts. Large colonies of acari were present in the infra-orbital fossæ, inguinal folds and on the perineum.

13.8.35. Only a few solitary acari were found in the crusts on the body, on the perineum and in the inguinal folds. Active colonies were present in the infra-orbital fossæ. The mites from these, about 20 in number, were transferred to a clean sheep, No. 17. The body was still covered with crusts, although the wool was growing.

22.8.35. A few acari, chiefly ovigerous females, were found on latent lesions under the crusts which had not lifted from the skin, on the back and in the inguinal folds and infra-orbital fossæ. The sheep was not showing very active signs of irritation. An abscess had developed on the left side.

13.9.35. The sheep was showing clinical symptoms of active scab.

4.10.35. The sheep was showing clinical symptoms of active scab.

6.11.35. Active lesions were found on the back. These consisted of moist areas surrounding the hard, dry crusts which had remained throughout the summer. Ovigerous females, nymphs and larvæ were found. The

infra-orbital fossæ and inguinal folds were free from acari. The wool, which showed a brownish tinge, had grown and many of the crusts had been lifted from the skin. All the clinical signs of scab were present.

Sheep No. 608.

Infected by artificial transference on 14.3.35. The disease developed rapidly and covered the whole body surface.

24.6.35. Attempts at carrying out observations on acari in cells on this sheep were still being made at this date. A latent lesion was present on the perineum between the crutch and the thigh of the right leg. One ovigerous female, which was not particularly active, was found on the lesion. The acari used in the cell work were taken from Sheep No. 340, on which the scab was active at this time.

1.7.35. *Right side.*—A small lesion was found on the costal region of the 10th–12th ribs. Two ovigerous females and one male were found. These may have escaped from a cell.

Perineum.—The latent lesion of 24.6.35 was still present but had not increased in size. Two ovigerous females, two larvae and one egg were found. No other lesions or acari were found.

8.7.35. *Right side.*—The latent lesion in the costal region had dried up and no acari were found.

Perineum.—The latent lesion on the perineum was still present. A few acari of all stages were found in the midst of the crusts.

Observations on acari in cells were discontinued after this date. Since 1.7.35 only acari found on the perineum of the sheep were used in the cells.

16.7.35. *Right side.*—Only a few dry crusts could be found on the site of the latent lesion of 24.6.35.

Perineum.—The latent lesion in the crutch appeared to have increased in size and a few acari of all stages except eggs were found.

The wool had grown considerably on the body and most of the crusts had disappeared from the skin, which was quite healthy. The sheep was not showing any symptoms of active scab.

22.7.35. The latent lesion on the perineum was still present. Only one ovigerous female was found.

1.8.35. The latent lesion on the perineum appeared to have dried up. Neither lesions nor acari were found anywhere on the sheep.

13.8.35. Neither lesions nor acari were found.

22.8.35. Neither lesions nor acari were found.

28.8.35. Neither lesions nor acari were found.

25.9.35. Neither lesions nor acari were found.

4.10. Neither lesions nor acari were found.

9.10.35. The sheep was reinfected by artificial transference. The sheep was never isolated during the whole of the summer, but was run in a pen with Sheep No. 11 and No. 340, both of which were harbouring acari.

Sheep No. 12.

This sheep had been used in an experimental dipping trial on February 4th to 16th, 1935. On 23.4.35 it was found to have active scab. After dipping, the wool had commenced to grow and many of the crusts on the body were lifted completely from the skin.

24.6.35. *Right side.*—An area of crusts still remained around the withers. In these solitary ovigerous females were found on latent lesions.

Left side.—Neither acari nor lesions were found.

Perineum.—A small latent lesion was found on which were acari in all stages.

1.7.35. *Right side.*—A few acari in all stages except eggs were found among the dry crusts behind the withers.

Left side.—Two latent lesions were found midway along the back and two to three inches from the spine. Three ovigerous females and three larvæ were present.

Perineum.—The latent lesion of 24.6.35 was still present, without any appreciable increase in size. Acari were still present.

8.7.35. *Right side.*—Acari were still present among the dry crusts of the withers. These were not in large numbers and consisted chiefly of ovigerous females. No eggs were found.

Left side.—The latent lesions were found as on 1.7.35. On these were only ovigerous females.

Perineum.—The latent lesion was the same as on 1.7.35. Four ovigerous females were found.

16.7.35. *Right side.*—Latent lesions and acari were found in the crusts behind the withers. The acari were not numerous and consisted chiefly of ovigerous females, but no eggs.

Left side.—Lesions were found on the same places as on 1.7.35. The acari were chiefly ovigerous females. A few immature stages were found.

Perineum.—The lesion was the same as on 8.7.35. Only ovigerous females were found.

22.7.35. *Right side.*—The latent lesions of 16.7.35 were still present. Only ovigerous females were found.

Left side.—The latent lesions were present as on 16.7.35 and ovigerous females were found.

Perineum.—Two small moist lesions were found on which were ovigerous females, a few immature stages and no eggs.

1.8.35. Acari were found on numerous latent lesions in the dry crusts or scurf on both sides of the body in the neighbourhood of the spine. Except for one larva and one egg, all acari observed were ovigerous females.

Perineum.—The two lesions of 22.7.35 were still present but had not increased in size. Ovigerous females were found.

The wool had grown completely and the sheep was not showing any active symptoms of scab. Fig. 8 (page 168) shows the condition of this sheep on 8.8.35.

9.8.35 and 19.8.35. The sheep was double-dipped on 9.8.35 and 19.8.35 in an approved arsenic sulphur powder dip at full and half strength. After dipping, the sheep was isolated with No. 609.

22.8.35. One suspicious lesion was present on the perineum. No acari were found.

28.8.35. Some moisture was present under the crusts on the scrotum and on the perineum. The sheep was inclined to show the labial reflex when these crusts were removed. No acari were found.

25.9.35. Neither acari nor lesions were found on any part of the sheep, including the infra-orbital fossæ and inguinal folds.

6.11.35. No acari, lesions or crusts were found on the sheep. The scab would appear to have been cured.

Sheep No. 609.

The sheep was infected on 30.3.35 and the scab progressed fairly actively over the whole body.

24.6.35. *Right side.*—Two latent lesions were present on the mid-flank and just in front of the hip. Ovigerous females and larvæ were found.

Left side.—On one latent lesion just in front of the mid-flank, two ovigerous females, one pubescent female and one larva were found.

1.7.35. *Right side.*—A suspicious latent lesion was found near the shoulder, but no acari were observed. The lesions of 24.6.35 appeared to have dried up.

One male was found in the right inguinal fold.

Left side.—The lesion of 24.6.35 appeared to have dried up. No acari were found. One ovigerous female was found in the left inguinal fold.

8.7.35. *Right side.*—Neither acari nor lesions were found.

Left side.—Neither acari nor lesions were found.

No acari were found in the inguinal folds.

16.7.35. *Right side.*—The lesions of 1.7.35 had dried up completely. No other lesions or acari were found.

Left side.—Two dry lesions, one three inches and the other six inches in extent, were found behind the shoulders. The sheep was inclined to show the labial reflex when these parts were handled.

22.7.35. *Right side.*—No acari were found.

Left side.—Three latent lesions were found behind the shoulders. Two of the lesions were on the same sites as on 16.7.35. The third was near the hip. One ovigerous female was found on each lesion.

1.8.35. *Left side.*—The latent lesions of 22.7.35 were still present and had not increased in size. One dead ovigerous female and two nymphs were present on the lesion nearest the shoulder and one ovigerous female on each of the other lesions. No acari were observed in the infra-orbital fossæ or inguinal folds.

9.8.35 and 19.8.35. The sheep was double-dipped in an approved arsenic sulphur powder dip on 9.8.35 and 19.8.35 at full and half-strength. After dipping it was isolated with No. 12.

22.8.35. Neither acari nor lesions were found.

28.8.35. Neither acari nor lesions were found.

25.9.35. Neither acari nor lesions were found.

6.11.35. Neither acari nor lesions were found.

Sheep No. 11.

This sheep was infected on 20.3.35. The scab progressed fairly rapidly over the body, but not over the head or limbs.

24.6.35. The sheep's body was covered with crusts but the wool had commenced to grow. The skin was very much wrinkled, especially around the withers and on the back.

Right side.—Semi-latent lesions were found in the wrinkles on the withers. Twenty to 30 acari, including coupled pairs and all stages, except eggs, were found. A similar lesion was found at the costal region of the third to fourth ribs, where all stages of acari were present except eggs.

Left side.—A lesion in the wrinkles of the skin, similar to those on the right side, was observed. There was moisture in the folds of the wrinkles. Thirty to 40 acari of all stages, including nine eggs, were found. There was also a lesion at the costal region of the eighth to ninth ribs, acari being present.

Perineum.—A small moist lesion below the vulva, on which was one ovigerous female, was observed. The sheep was showing active signs of irritation.

1.7.35. *Right side.*—Three latent lesions were found; one behind the withers as on 24.6.35, one two inches behind the first and a third near the spine in the lumbar region. In none of these lesions was the moist area more than a quarter of an inch in diameter and the crusted area more than six inches in diameter. A few acari of all stages except eggs were found on the lesions, lying on the under-surfaces of the crusts. The crusts on removal left the skin moist.

Left side.—The lesions of 24.6.35 appeared to have died out.

Perineum.—Two latent lesions were present on either side of the central line of the perineum; two ovigerous females and a few immature stages, but no eggs, were found on each.

Infra-orbital fossæ. Colonies of acari which included all stages were present in these fossæ. The ovigerous females were, however, the most numerous. The fossæ had not previously been examined.

8.7.35. *Right side.*—The two most anterior lesions appeared to have dried up. On the lumbar lesion one rather comatose ovigerous female was found.

Left side.—Neither lesions nor acari were found.

Perineum.—The two latent lesions were still present, on one of which was one ovigerous female and on the other one ovigerous female and a larva.

Infra-orbital fossæ.—The colonies were still present.

16.7.35. *Right side.*—The latent lesion in the lumbar region was still present with one ovigerous female.

Left side.—Neither lesions nor acari were found.

Perineum. The two latent lesions, as on 8.7.35, were still present. Two ovigerous females and one male were found.

Infra-orbital fossæ. Right.—Acari, including immature stages, were still present, but no eggs were noted.

Left.—Six ovigerous females were present.

22.7.35. *Right side.*—Not examined owing to the presence of an abscess which had formed on the left side and which was under treatment.

Perineum.—The two lesions appeared no larger than on 16.7.35. Two ovigerous females and one larva were found.

Infra-orbital fossæ.—Acari were still present in these fossæ, but they consisted chiefly of ovigerous females.

1.8.35. *Right side.*—One dead ovigerous female and one larva were found on the first lesion behind the withers (see 1.7.35). A new latent lesion had appeared in front of the third lesion of 1.7.35 and on it was one ovigerous female.

Perineum.—The two lesions appeared larger than on 22.7.35. Six ovigerous females and one pair *in copula* were found.

Infra-orbital fossæ.—Acari, chiefly ovigerous females, were found in the fossæ.

14.8.35. Sheep Nos. 20, 98, 279, 439 and 607 were infected with acari (five to six ovigerous females to each) taken from the infra-orbital fossæ and perineum of this sheep.

21.8.35. The sheep was destroyed, owing to debility arising from the abscess on the left side. At the time of death, the wool had grown and the sheep had not shown any symptoms of active scab since the beginning of July.

Sheep No. 17.

14.8.35. This sheep was infected with about one dozen ovigerous females and a few immature acari from the infra-orbital fossæ of Sheep No. 340.

22.8.35. An active crusted lesion had formed on the withers near to the point of transference. The lesion was about the size of a shilling. One ovigerous female, one nymph and three eggs were found.

28.8.35. The lesion had not increased in size and a few acari were present.

25.9.35. No very appreciable increase in size of the lesion had taken place. Four ovigerous females and one larva were found.

4.10.35. Active scab, covering an area of about one square foot, was present on the withers, shoulders and spine. Acari were plentiful and ten ovigerous females and three coupled pairs were transferred to other sheep. This activity of the scab occurred after a week of cold weather.

23.10.35. The active scab had increased to cover an area from in front of the withers to the lumbar region and down to the costal areas. During the period 4.10.35 to 23.10.35, 535 ovigerous females and 158 coupled pairs were removed for transference to other sheep.

Sheep Nos. 20, 98, 279, 439 *and* 607.

These sheep were infected with five or six ovigerous females from the infra-orbital fossæ and perineum of Sheep No. 11 on 14.8.35.

22.8.35. All the sheep were showing small lesions at the point of transference. The acari were chiefly ovigerous females.

28.8.35. The lesions had not increased since the last inspection. It was doubtful if there were any live acari on Sheep No. 98.

25.9.35. Sheep Nos. 279 and 439 were showing lesions and acari were found, but the immature stages were few. On Sheep No. 20 only two ovigerous females and one male were found. On Sheep Nos. 98 and 607 the lesions had dried up.

4.10.35. Sheep Nos. 279 and 439 had active scab on the withers, although it had not progressed rapidly. On Sheep No. 20 only one male was found. Sheep Nos. 98 and 607 were negative. These latter three were reinfected from No. 17.

(*b*) *Discussion.*

From the foregoing series of records it is possible to form some general conceptions of the nature and causes of latency in scab and the salient features of the series may, with advantage, be summarised in brief as a preliminary to the discussion of their bearing on the problem. The general implications of the latency phenomenon in relation to control measures will be discussed in a later section.

(1) The onset of latency coincides with the commencement of the warmer weather of the year and ceases on the return of cold weather.

(2) In the first eleven cases recorded, with the exception of No. 340, the sheep were affected with active scab in the winter or early spring. In these ten instances the infection spontaneously decreased in severity in the summer. No. 340 presents certain peculiarities and will be referred to later. (See No. 10.)

(3) In three (Nos. 24, 36 and 608) of the eleven cases, the spontaneous recovery was complete. In three other cases the sheep were dipped or destroyed before the autumnal relapse occurred and in the remaining five

cases the summer recovery was followed by a relapse in the succeeding winter.

(4) In all the eleven cases acari could be found on the sheep after the infection had become latent. In those which relapsed, the acari were present in small numbers right through the latent period and were frequently found in the infra-orbital fossæ and inguinal folds and in certain cases were confined to these parts.

(5) In some cases the numbers of acari showed a marked reduction as early as March. Recrudescence of activity occurred from August to November.

(6) During the latent phase there may be a total absence of the clinical symptoms of the disease, despite the fact that acari may be found by careful searching.

(7) In the latent phase, all stages of acari occur, but there is a great preponderance of fasting and almost non-ovipositing females.

(8) There appears to be no appreciable lengthening of the duration of the life-cycle of individual acari during the latent phase.

(9) Acari taken from sheep with latent scab when placed on clean sheep are capable of producing an active infection.

(10) Sheep No. 340 was infected with acari in the late spring, when the weather conditions at the time were unseasonably cold, producing active scab immediately. Six other cases were infected in the summer. The scab was established on three but did not progress rapidly until October. On the other three the infection failed to develop and died out completely.

(11) The recrudescence of the disease in the winter, after two latent periods (Sheep Nos. 3 and 340), is not so virulent as winter scab on sheep which have had no previous infection.

The above summary of the records of suppressed or latent scab definitely establishes the fact that in all cases where active scab has become latent in the summer, to revert to the active phase in the following winter, acari are continuously present on the body of the sheep throughout the latent period. Although ovigerous females predominate, any one of the life phases may be encountered. Stockman and Berry (1913)[10] have published microphotographs of ovigerous females showing unlaid eggs which contain larvæ. The writer has examined large numbers of ovigerous females at all times of the year, including females from latent cases, but has been unable to observe this phenomenon. Some females were found to contain eggs, but these were quite normal and undeveloped.

There appears to be no evidence of the occurrence of a resting phase, or that the acari burrow into the skin or tissues.

The analysis serves to emphasise certain characteristics of suppressed scab in the summer. Firstly, there is a definite cessation in the activity of the acari in the summer. At this season the infection is not progressive because the inactive mites remain confined to certain parts where they find shelter. These sites of latent infection were (*a*) naturally protected areas, such as the infra-orbital fossæ and inguinal folds; (*b*) small inactive lesions scattered on the body surface. During the latent or suppressed

phase a sheep may be harbouring acari on one or all of such sites. On Sheep Nos. 3 and 409 the infection in 1933 was confined to the inguinal folds. In 1934, when the skin was in a very wrinkled condition, the infection was scattered on the body surface, although No. 3 had acari also in the left inguinal fold. In 1934, Nos. 11 and 340 had acari in the infra-orbital fossæ and on the body. In the remaining sheep the infection was scattered on the body surface.

With the cessation of the latent period, however, there is a tendency for the acari to leave the naturally protected sites and to migrate generally over the body surface. At this time also the latent lesions on the body again become active, due to the centrifugal migration of the mites, which feed and so create a zone of active scab. At first the process proceeds slowly but it becomes more rapid with the advent of cold weather in October and November.

Secondly, there is no intrinsic difference between the acari causing scab in the winter and in the summer. Artificial infection will produce active and rapidly progressive scab in the winter, or active but very slowly progressive scab in the summer. Sheep No. 340 is of interest in this connection, in that the infection occurred in late spring under conditions of low temperature; the infection resulted in a rapidly progressive scab of the winter type which, with the subsequent change to warmer weather, became latent. Moreover, inactive mites taken from latent lesions will produce active scab when transferred to clean sheep (Sheep Nos. 279 and 439 infected from No. 11 and Sheep No. 17 infected from No. 340). Conversely, acari from an active lesion, when placed on a sheep with latent scab, produced a slowly progressive summer type of lesion (Lamb No. 2—24.7.33).

Thirdly, there is no evidence of suspension, with a resting phase in one particular stage, of the life-cycle during the latent phase. The life-cycle from egg to ovigerous female would appear to proceed normally, but the rate of oviposition appears to be much reduced. This reduction is indicated by the high ratio of adults to immature stages and eggs observed.

With regard to the factors controlling latency, the evidence permits of certain conclusions being drawn. The analysis of the results on pp. 198 and 199 suggests that climatic conditions play an important part. As has already been stated, latency coincides with the onset of warmer temperatures, endures through the period of warm weather and relapses to activity as the temperature falls in August and September. Fig. 9 shows the average half-monthly minimum temperatures for the years 1933 and 1934 at Little Gaddesden. It will be noticed that the minimum temperatures were rising at the end of February and continued to rise until July. From March onwards a difficulty was experienced in finding acari on the sheep and in April and May

FIG. 9.

Minimum Temperatures: Degrees Fahrenheit.

the infection had become quite inactive. From the end of August or the beginning of September the minimum temperature begins to fall and the scab begins to show signs of resumed activity.

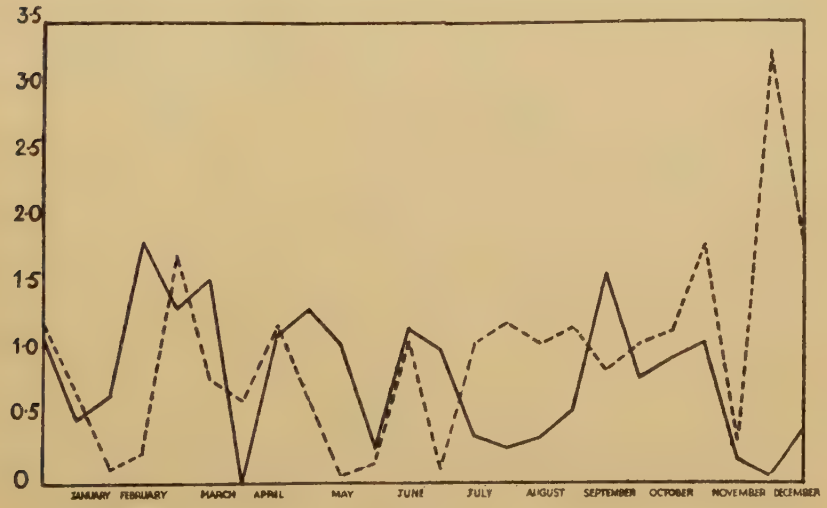


FIG. 10.

Rainfall in inches.

Fig. 10 shows the total rainfall in half-monthly periods for the years 1933 and 1934 at Little Gaddesden. There is a popular belief that scab is more active in rainy weather. Here there does not seem to be any correlation, since both curves rise during March and April when the scab was becoming latent. There is also a rise in June and July in the middle of the latency period. Little rain fell in December 1933 and February 1934, but the scab was very active.

Table IX (page 178) shows the effect of temperature on the rate of oviposition. The higher the minimum temperature, the lower is the rate of oviposition and the longer is the life of the ovigerous female. That oviposition occurs, at least to a limited extent during latency, is shown by the finding of immature stages and occasionally eggs on latent lesions, also some females taken from latent lesions have been observed to contain eggs. At the same time, the rate of oviposition must be greatly reduced, as in all instances the ovigerous females equalled or outnumbered the immature stages.

A second factor which may influence the rate of oviposition is the humidity at the skin's surface. It was noticed that in every case, when latency commenced, the affected areas of skin were more or less denuded of wool. This condition must create a much steeper moisture gradient at the skin surface and consequently a lower degree of humidity in the immediate environment of the mites. By the time the relapse had occurred in the autumn new wool had grown on the bare patches.

There would appear to be a third factor which also influences the rate of oviposition. In every case, except No. 340, all the sheep which had latent scab were those infected either during the winter or early spring (before April) and in all cases the scab had developed over almost the whole of the body surface, leaving the skin crusted, wrinkled and indurated. In the autumn, when the scab became active again, the wool had grown and the skin regained its normal condition to a large extent. At the same time, on sheep which had had more than one attack of active scab (Nos. 3 and 409), the skin did not regain its normal condition so readily at the end of the second latent phase, neither did the scab progress so rapidly after the relapse to activity. These factors appear to indicate the probability of some reaction on the part of the skin, which renders it distasteful to the acari and tends to reduce the rate of oviposition. This is supported by the following observation :—

Ovigerous females have been taken from sheep with latent lesions and put in cells on clean or slightly scab-affected sheep. Here they have laid eggs which have developed through the various stages to the adult in little longer than the normal period.

The above description of latent scab shows that the apparent freedom of sheep from the disease in the summer is due to a reduction in the rate of multiplication of the acari, associated with

an increased mortality owing to unfavourable conditions. It would appear that there is some element of chance as to whether this reduction is carried so far as to lead to a total extermination of the acari and a complete recovery, or whether sufficient acari succeed in surviving through to the autumn to produce a relapse to active scab. That no intrinsic difference existed between those sheep which recovered and those which relapsed is clear from the fact that No. 608, after recovery, was susceptible to reinfection later. Further mites could be demonstrated on both groups (recovered and relapsing sheep) for some time after the onset of latency.

Of the five sheep infected from No. 11 (acari from the infra-orbital fossæ), three failed to develop active scab in the ensuing winter. These three cases may represent recovery after a mild infection of the summer type of active scab; on the other hand, the explanation may lie in either of the two following possible causes:—

(*a*) That the females laid so few eggs that only one sex was represented in the progeny, as on Sheep No. 20 (25.9.35);

or (*b*) That the females had lost their ability to oviposit before being transferred.

The following case illustrates the second possibility:—

On 26.9.33 an ovigerous female was taken from a latent lesion and placed in a cell on Sheep No. 3, in which the infection was just emerging from latency. The female lived until 24.10.33, when she died. During this time she fed occasionally but failed to lay a single egg.

(4) *Discussion on the General Clinical Aspect of Scab.*

As has been mentioned already, in its clinical aspect sheep scab may be divided into three phases:—

(*a*) Active scab in winter.

(*b*) Active scab in summer.

(*c*) Latent or suppressed scab in summer.

There is, however, a fourth phase, which has been noted during the observations at Little Gaddesden. On Sheep Nos. 3 and 409 it was observed that in the winter of 1934, after the scab had been through two latent phases, it did not become as active as on relatively clean sheep. Also, at the end of the second latent phase the skin did not recover its normal condition so completely. This type of scab might almost be termed *slowly progressive, or chronic winter scab.*

Assuming that the hypothesis discussed in the previous sections is correct—that the complex effects of climatic conditions and skin reaction influence the rate of oviposition of the ovigerous females and so, the activity of the scab—it is of interest to compare this activity in the various seasons.

(*a*) *Active scab in winter.*—The cold climatic conditions are favourable

to a high rate of oviposition. The skin reaction is also favourable. This type of scab is usually found on clean sheep recently infected.

(*b*) *Latent or suppressed scab in summer.*—The climatic conditions, *i.e.*, warm weather, are unfavourable and so also is the skin reaction, as latency was found only in sheep which had had a severe infection of scab in the previous winter and whose bodies had been almost completely covered with lesions.

(*c*) *Active scab in the summer.*—Notwithstanding the prevalence of warm weather, the skin reaction is favourable. This type of scab occurs in clean sheep infected in the late spring.

(*d*) *Slowly progressive or chronic winter scab.*—In this form the climatic conditions (*i.e.*, cold weather) are favourable but the skin reaction is unfavourable. This type of scab occurred on sheep whose skins had not completely recovered during the second latent phase.

It would appear that the seasonal variations are a matter purely of degree and not one of any difference in the type of the disease. The clinical symptom of pruritus is dependent on the relative number of acari feeding on the skin, which again depends on the rate of oviposition.

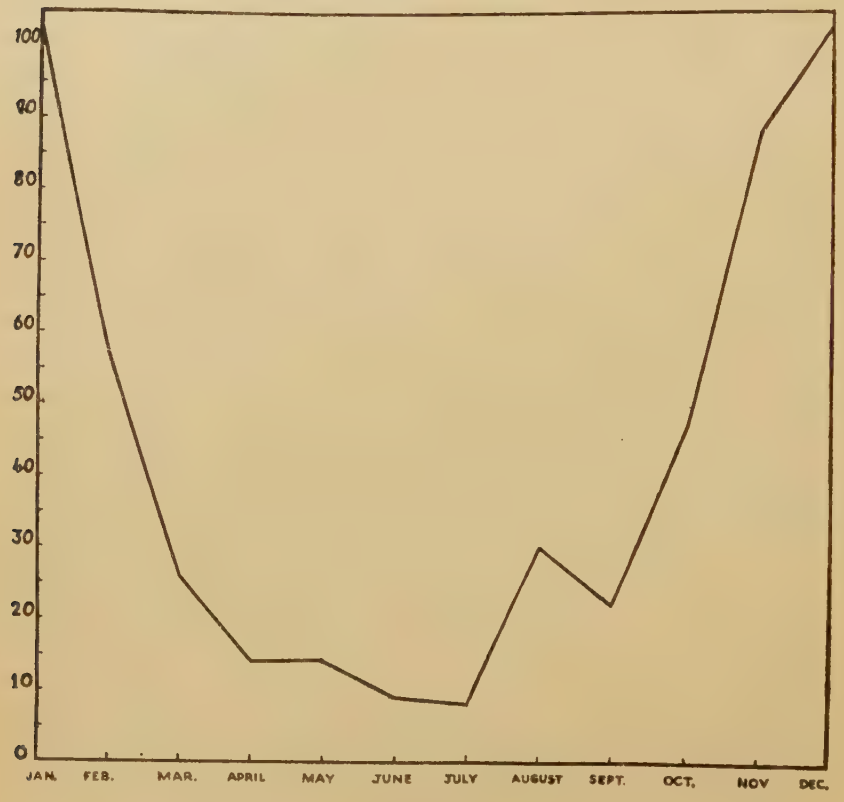


Fig. 11.

Number of outbreaks of scale.

(5) *Seasonal Variation of Scab Incidence in the Field.*

It is of interest to compare the above observations on animals under experimental conditions with the records of the occurrence of scab in the field.

Fig. 11 is a composite graph showing, in monthly periods, the number of outbreaks of sheep scab in Great Britain based on the returns of the Ministry of Agriculture, as published in the *Veterinary Record* for the years 1929 to 1934. It has been stated that on the Research Station sheep scab develops much more rapidly in the winter than in the summer months, due to the greater activity of the ovigerous females and, although scab may be active in the summer, it is of much slower development. It will be seen in the graph that a similar seasonal variation occurs in the field; the greatest number of outbreaks are reported in January and December and the least in July.

There is a marked rise in the month of August and then a fall in September before the curve rises again. A probable explanation of the August increase in incidence is that, as this month is the period during which statutory dippings are carried out, the sheep on hill pastures come under closer supervision than that to which they have been subjected during the rest of the year. In many cases, owing to the slow rate of the spread of the disease in the summer months, it is possible that the sheep had been affected for some considerable time, but that the disease had escaped observation.

The graph would indicate that latency is not merely a phenomenon observed in experimental animals. The very marked drop in the number of outbreaks of scab in summer, followed by so great an increase in the winter months, could hardly be due merely to the slowing down of the disease, and indicates that in many cases practically complete suppression of activity—true latency—has occurred, involving, in most cases, the apparent disappearance of all traces of scab. Thus, in some of the experimental sheep, where the acari have been confined to the infra-orbital fossæ, the inguinal folds or the perineum, it would have been possible for these sheep to have been sold in good faith without the presence of the disease being suspected. Should these sheep have been sold into different parts of the country, they would each have been a potential source of a fresh outbreak of scab.

IV.—Bionomics in Relation to Control Measures.

Table X shows that the complete life-cycle of the *Psoroptes communis* var. *ovis* from egg to egg is usually completed in eleven days, with ten days as the shortest period and twelve as the longest. In every instance the period from the appearance of the larva to the subsequent egg was eight days, except in the twelve days life-cycle, when it was nine days. The time that elapses

between the first appearance of the larva and the subsequent oviposition is very important in relation to measures of control by dipping and the selection of the optimum dipping interval. There is, at the present time, no dip known which can be relied upon to sterilise the eggs and for this reason a second dipping must be given with the object of killing acari which may have hatched after the first dipping, before they have reached the adult stage and commenced ovipositing. The usual practice in most countries is to give this second dipping ten to 14 days after the first. In theory this practice might appear questionable, as eggs which hatch out, even up to two days after the first dipping, may have developed to females and have commenced to oviposit before the second dipping, even if this takes place after a ten-day interval. This assumes that there are males present when the females reach the pubescent stage. If, however, all the males have been killed at the first dipping, the females have to wait for the newly-hatched males. These require two and a half to three days longer than the pubescent females to reach maturity and so the interval between larva and egg will be prolonged from ten and a half to eleven days.

In actual practice there is another factor which has an important bearing on this problem, and this is the amount of dip which remains in the fleece after the first dipping. If sufficient remains in the fleece to kill any larvæ which may hatch out during the first two days, then the ten-day interval of dipping is satisfactory, as any larvæ hatching out after that time will hardly have reached the ovipositing stage before the second dipping. The amount of dip remaining in the fleece after the first dipping is very dependent on rainfall after dipping. That there is sufficient dip left under favourable conditions is shown in the following experiment :—

(1) Two sheep, Nos. 401 and 412, were dipped in a full-strength bath of an approved arsenic-sulphur powder dip on 21.12.33. The sheep were in long-fleece and one minute's immersion was given.

(2) On the same date, two sheep, Nos. 394 and 423, were dipped in a full-strength bath of an approved carbolic fluid sheep dip. The sheep were in full fleece and one minute's immersion was given.

On alternate weeks one sheep in each pair was infected with a large number of acari of all stages. Thus No. 394 and No. 412 were infected on 28.12.33, No. 401 and No. 423 on 3.1.34 and No. 394 and No. 412 a second time on 12.1.34, and so on. By this means each sheep was under observation 14 days after the first transference, before the second occurred on the same sheep. After the third attempt (21 days) No. 394 (carbolic dip) became infected with scab, whilst the arsenic-sulphur dipped sheep were not infected until 12.2.34 (53 days after the dipping). That the acari had been killed by the dip was shown by the finding of dead acari. As has been stated, this experiment was carried out under ideal conditions, since the sheep were housed the whole time and not exposed to rain.

In the histories of sheep given in the section on latency, it will be noticed that Sheep Nos. 12 and 609 were dipped whilst the scab was latent and while they were at the time harbouring acari. The double-dipping was effective in curing the scab. The acari, however, were only present on the sides and the perineum, where the dip had easy access to them. If acari are present in the infra-orbital fossæ, the inguinal folds, the scrotum of the wether and possibly the tail, an ordinary dipping without hand-dressing is less likely to be effective. Where large numbers of animals have to be treated, hand-dressing would entail an excessive amount of labour and expense, especially in hill flocks. As the writer has already pointed out, the acari show a tendency to migrate from these places of concealment in the autumn and thus become more accessible to dipping fluids and he is of the opinion that dipping at this time of the year would give a better control of sheep scab than does the present summer dipping.

V.—Conclusions.

(*a*) Under the climatic conditions of Great Britain, the life-cycle of *Psoroptes communis* var. *ovis* is usually completed in eleven days, with a minimum period of ten days and a maximum period of twelve days.

(*b*) The duration of the life-cycle of the acarus, from the first appearance of the larva to the commencement of oviposition, is usually eight days (maximum nine days) but, since the male requires two and a half to three days longer than the female to reach the copulatory stage, unless males of a previous generation are present when the females reach the pubescent stage, the period will be prolonged to ten and a half or eleven days.

(*c*) The number of eggs laid by ovigerous females shows relatively little individual variation, but the rate of oviposition shows considerable variation.

(*d*) The variation in the rate of oviposition appears to be dependent on :—

(1) Temperature and possibly humidity.
(2) Some form of skin reaction inimical to the parasite.

(*e*) Sheep which are heavily infected in winter recover spontaneously in the summer. This recovery may be complete, or more frequently is followed by a relapse in the following autumn, the scab having remained latent through the summer. In all such cases acari are continuously present throughout the latent period, whereas in cases of complete recovery the acari have failed to survive the complete latent period.

(*f*) During the summer there is a very marked reduction in the number of reported outbreaks of sheep scab, undoubtedly due to the reduced activities of the acari.

(*g*) Where the infection is contracted in the spring or summer the scab may remain active, but progress is very slow until the autumn.

(*h*) It is possible for sheep to harbour a few acari in the summer without showing any clinical symptoms of the disease.

(*i*) In cases of latent scab the most important sites of the body on which acari may be present are the infra-orbital fossæ, the inguinal folds, the scrotum of the wether and the tail. In autumn and winter the acari tend to migrate from these parts to the general body-surface. An autumn statutory dipping should, therefore, be more effective against scab than a summer dipping.

(*j*) On sheep in which the scab is latent in the summer the disease becomes active in the autumn and so a healthy flock may be infected by sheep which are apparently clean when introduced in the late summer. This is the probable explanation for the outbreaks of sheep scab in flocks which have not been in contact with any known source of infection.

(*k*) The most favourable time of the year in which to dip sheep for the control of sheep scab is the autumn, when the acari have migrated from the sheltered local sites to the general skin surface of the body.

References.

[1]Walz, G. H. (1809). Natur und Behandlung der Schaf-Räude. Stuttgart. Cited by Tessier, Fournier, Niemann, Youatt, Gerlach and Fürstenberg.

[2]Hering, E. (1835). *Amtlicher Ber.* (12*te*). *Versamml. deutscher Naturf. v. Aerzte zu Stuttgart im September*, 1834. 135.

[3]Hertwig, C. H. (1835). Ueber die Krätz-oder Räudemilben. *Mag. ges. Thierh.* I (2), Berlin, 165-198, pl. 3, figs. 6 and 7.

[4]Delafond, O., and Bourguignon, H. (1856). Résultats des recherches entreprises sur la gale du mouton. *Rec. Méd. Vetér.*, III (4), Paris, 98-114, 171-182 and 321-335.

[5]Gerlach, A. C. (1857). Krätze und Räude, Berlin, 178 pp., 8 pls., 44 figs.

[6]Stockman, S. (1909). *Ann. Rept. Proc. under Dis. Animals Acts*, London, 10-14.

[7]——. (1910). Some points in the Epizootiology of Sheep Scab in relation to Eradication. *Journ. Comp. Path. and Therap.*, xxxiii (4), London, 303-314.

[8]——. (1911). *Ann. Rept. Proc. under Dis. Animals Acts*, London, 22-30.

[9]Stockman, S., and Berry, A. H. (1913). The Psoroptes communis ovis. Some observations on Ova and Ovipositing. *Journ. Comp. Path. and Therap.*, xxxvi (1), London, 45-50, 3 figs.

[10]Shilston, A. W. (1915). Sheep Scab. Observations on the Life-history of *Psoroptes communis* var. *ovis*, and some points connected with the epizootiology of the disease in South Africa. *3rd and 4th Repts. Dir. Vet. Res., Union of South Africa, Dept. of Agric.*, Pretoria, 69-98.

Acknowledgments.

I wish to express my sincere gratitude to Dr. L. E. Robinson and Mr. W. C. Prudames and to all my other colleagues of the Cooper Technical Bureau for their encouragement and assistance given to me whilst carrying out this work and in the preparation of this paper.

I am greatly indebted to Dr. Robinson for his kindness in permitting me to make use of his line-drawing of the *Psoroptes communis* var. *ovis*, as illustrated in Plate I ; and to Major Wykeham-Musgrave for the use of his meteorological records, from which the data for the graphs in Figs. 9 and 10 were abstracted.

My thanks are also due to Messrs. Cooper, McDougall and Robertson, Ltd., Berkhamsted, for providing me with the necessary facilities for this work.

NEMATODES IN SHEEP IN ICELAND. EXPERIENCE WITH CARBON TETRACHLORIDE DRENCHES.

By Professor Niels Dungal and Gudm. Gislason,
Department of Pathology, University of Reykjavik.

For a long time the farmers in Iceland have spoken about a worm-disease in sheep, without having any clear conception as to the ætiological factors. Many ailments in sheep have been attributed to nematodes without support of any research work.

The chief symptoms which have been claimed to be caused by worms are on the one side wasting and bad condition in spite of sufficient food, on the other side acute or chronic diarrhœa showing a more or less high percentage of mortality.

In later years sheep breeding in Iceland was severely troubled by some unknown diseases, which most people supposed to be caused by worms. The symptoms of these diseases were chiefly the two above mentioned, namely long-standing wasting without any apparent cause and on the other side acute or chronic diarrhœa, which was noticed chiefly during the first spring months and in the latter half of the winter.

In most farms in Iceland sheep are kept in houses during one to three months in winter-time, but are grazed as much as possible also during winter. In summer sheep from all parts of the country are driven into the rich pastures in the inner part of the country, from which they usually return in excellent condition in autumn.

After 1930 an increase was noticed in the sheep mortality on the whole. The sheep were difficult to keep in condition during winter, and in many places they were noticed to return in poor condition from the summer pastures. In the following years, 1931 and 1932, complaints were heard from all parts of the country that the sheep breeding was not paying because of the enormous losses suffered from what was considered to be worm disease. The prospect for the farming industry had in 1933 grown so serious that the Government decided to intervene and asked us to take up research work in this matter in order to discover the cause and if possible prevent the impending disaster which threatened the farming part of the population. During the slaughtering season in autumn 1933 one of us (N.D.) investigated worms in the intestinal tract of 102 sheep from different places in the south-west part of the country, where this disease had been very prominent. This investigation, which only applied to nematodes in the abomasum and small intestine, gave the following results: —

Table I.

	Abomasum.	*Small-intestine.*	
	Ostertagia circumcincta	*Nematodirus filicollis.*	*Bunostomum trigonocephalum.*
Were found in	84 per cent.	66 per cent.	78 per cent.

In the large intestines only an occasional *Chabertia ovina* was found, and they were thought to be of negligible importance.

The worm which appeared to be the most dangerous parasite was *Bunostomum trigonocephalum*. Where this worm was in considerable numbers the small intestine was densely beset with small petechiæ, which were easily seen from outside through the serosa of the gut, and the more easily so because in every case where this infestation was considerable the mesenteric fat was scanty and in a large number of sheep we found the intestines practically devoid of fat, which was remarkable as this investigation was carried out in the autumn months, October and November, when the sheep as a rule are in very good condition. In some sheep the colour of the small intestine was black-red from old and new hæmorrhages caused by *Bunostomum*. These changes were usually seen beginning about one to two yards below the pylorus and extending more or less down the jejunum.

Nematodirus filicollis was in some cases found in considerable numbers, and then usually in smaller or greater heaps in the grey mucus of the jejunum. We could not assign any particular pathological changes to this nematode, yet we found it in two cases in enormous numbers where a lamb had died after a short acute attack of diarrhœa and were inclined to attribute the death to this worm.

Ostertagia circumcincta seemed to be almost a normal inhabitant of the sheeps' abomasum and usually was found in enormous numbers there. We did not find any particular changes in the abomasum which we could assign to this worm, but we shall refer later to the invasion of *Ostertagia* larvæ into the mucosa of the stomach wall.

Our impression from this investigation was that the chief malefactor was the *Bunostomum*, as the poor condition in the sheep was usually found to go parallel with the numbers of petechiæ caused by this worm in the small intestine. We also investigated the lungs and bronchi for nematodes, but as the findings are to be recorded in another paper we shall not refer to them here. We only wish to mention that the poor condition of the sheep did not seem to be in any considerable degree attributable to lungworm infestation.

Experiments to Combat Intestinal Nematodes.

Having our attention chiefly concentrated on the *Bunostomum*, we began the same autumn to make some experiments in treat-

ment. We secured 40 sheep in very poor condition, showing all symptoms of what we thought to be *Bunostomum* infestation, and tried different remedies which were thought to be of value against nematodes. The preparations we tried were :—

Chenopodium oil.
Cooper's arsenic tablets (each containing 0·027 grammes arsenic).
Copper sulphate (1 per cent. water solution).
Tobacco leaves.
Carbon tetrachloride.

Experiment with Arsenic Tablets.—Two sheep were dosed with four tablets each with two days interval and given no water. Six days after the last dose they were killed. *Result.*—Great numbers of living *Bunostomum* and *Ostertagia*.

Experiment with Copper Sulphate, 1 per cent.—Two sheep were dosed with 200 c.c. of this solution twice with three days interval. The sheep were killed seven days after the second dose. *Result.*—Living *Nematodirus* and great numbers of living *Bunostomum* in small intestine, and living *Ostertagia* were found in abomasum.

Experiments with Chenopodium Oil gave quite negative results.

Experiment with Tobacco Leaves.—On October 26th one sheep was dosed with 20 grammes of tobacco leaves. Slaughtered October 30th. *Result.*—*Ostertagia* + living. *Nematodirus* none. *Bunostomum* were found in small intestine in considerable numbers but about half of them were dead.

Experiments with Carbon Tetrachloride.

	Sheep Number.	*Number of Capsules.*	*Ostertagia.*	*Nematodirus.*	*Bunostomum.*
Oct. 26th	3	1	O	O	++
	1	2	+ (immobile)	+ (immobile)	+ (immobile)
	9	3	O	+ (immobile)	+ (many dead and most immobile)
	33	1 & 2	++ (almost all immobile but most shrunken)	+ (most immobile)	+ (most dead, a few mobile)
	73	2 & 2 (on consecutive days)	(+) (only vert few and immobile)	+ (immobile)	+ (immobile, almost all dead, only a few moving slightly)

These sheep which were dosed on October 26th with capsules containing 1 c.c. CCl_4 were given neither food nor water during the experiment and were slaughtered on October 30th. Nos. 33

and 73 were dosed twice on consecutive days, one with 1 c.c. and the other with 2 c.c. This experiment showed that a good result might be expected from carbon tetrachloride. Although the experiment with tobacco leaves also was promising, we did not wish to conduct those experiments further, as carbon tetrachloride was much more economical in practice and promised to be more effective than the tobacco leaves, which we in this one instance applied in a very big dose.

Further Experiments with Carbon Tetrachloride Capsules of 1 *c.c.*—Six sheep dosed November 26th, 1933, killed December 5th, 1933 :—

Sheep Number.	*Number of Capsules.*	*Ostertagia.*	*Nematodirus.*	*Bunostomum.*
69	5*	+ (living, few)	+ (living)	+ (immobile bloodless)
14	5*	O (living larvæ)	O	O
33	5 × 1*	O	+	O
67	5 × 2*	O	O (living larvæ)	I (bloodless)
88	5	+ (few)	+ (few)	+ (some living)
65	5×2	+ (few)	O (many larvæ)	O (old petechiæ)

*Two days' fasting.

These experiments seemed clearly to indicate that carbon tetrachloride is efficient against the intestinal nematodes, particularly the *Bunostomum*.

Experiments on Three Sheep and One Control with Carbon Tetrachloride Drench without Capsules.—During the winter, 1933-34, we wished to follow up some sheep drenched with carbon tetrachloride and for this purpose we made the following experiment :—

On December 14th three ewes were kept fasting for 48 hours and then drenched as follows :—

No. 1, 5 capsules CCl_4.

No. 2, 5 c.c. CCl_4 without capsules.

No. 3, 5×2 CCl_4 without capsules (on consecutive days).

No. 4, No drench.

These sheep were all kept together in the same stall and their weight was controlled fortnightly. The weight of the three drenched ones began at once to rise and during the following three months Nos. 1 and 2 had increased 4 kilogrammes in weight each, No. 3 showed a weight increase by 8 kilogrammes, whereas No. 4 (not drenched) showed the same weight as at the beginning of the experiment. (None of the ewes were in lamb.)

A fortnight after the drenching, the one sheep which had not

been treated and which before had looked the best of the four, was evidently lagging behind the others, which were improving rapidly in condition. When a month had passed the change was very pronounced, so that anyone could see the striking difference in appearance of the one not drenched compared with the others. Yet all the four sheep had the same food and were moving freely in the same stall.

As the demand for worm drench was very pressing we decided to distribute CCl_4 in capsules to farmers who wished to try them. We recommended them to give five capsules of 1 c.c. to full-grown sheep and four to lambs, and not to give any food during 48 hours before and 24 hours after the application, so that there was a total fasting of 72 hours. In autumn and winter, 1933-34, we distributed on the whole 186,000 capsules for approximately 40,000 sheep.

The results were uniform. The farmers who used the drug kept their sheep healthy, but those who did not suffered more or less severe losses in their stocks as in the previous years. The reports from the farmers showed many striking examples of the efficiency of the drug. In one part of the country, for instance, where sheep breeding was threatened with ruin from worm diseases, so that almost every sheep had returned in poor condition from the summer pastures, most of the farmers used CCl_4 capsules in autumn, 1933. Their lambs showed a sudden change from their poor condition into normal development and kept their health during the whole winter after one more dosing, whereas the few farmers who did not use this drug had enormous losses, particularly among their hoggs. Similar instances were related from different parts of the country where this drug had been tried.

Since the experience was unanimous from all over the country we expected a great increase in the use of the drug in the following winter. As the capsules were comparatively expensive we wished to employ the drug in a more economic way, and therefore we had the carbon tetrachloride delivered in tins and special drench tubes made which measured 5 c.c. Liquid paraffin was added in 10 per cent. volume to render the carbon tetrachloride less volatile and prevent the effects of inhalation.

During the winter, 1934-35, our Institute delivered 4,479 litres of this mixture, which corresponded to one dosage to 895,800 sheep. We recommended two dosings during the winter, one in December and another at the beginning of March and expected the sheep to keep their health by this method of administration.

We have received reports from all parts of the country on the results of our worm drench for the winter 1934-35. We obtained these by sending question-forms to the foreman of each of the 200 official districts of the country. We received answers from 100 of the farmers to whom the question-forms were sent. The three principal questions were the following :—

(1) What is the condition of the sheep to which our worm drench was administered ?

(2) Was there a notable difference in the health of sheep where worm drench was applied and where it was not?

(3) Was the health of sheep better than in previous years when worm drenches were not given?

In answer to the first question 89 per cent. reported that the sheep had been in good or excellent condition where the worm drench was applied.

To the second question 82 per cent. answered in the affirmative, that the sheep had been in definitely better condition where the worm drench was administered.

To the third question as to whether the health of sheep had been better than in previous years, 93 per cent. answered in the affirmative, that the difference had been definite or very great. Many mentioned particularly that the difference in the health of sheep could not have been caused by better food, as hay had been no better or worse than in previous years. The negative answers to this question were all from the north-eastern part of this country, where hay was very bad following a very wet summer.

Intoxications following Worm Drenching.—In very rare cases dangerous intoxications were observed following the administration of the drench. This happened chiefly where the sheep were fed soon after the drenching. Where our recommendation was followed, to keep the sheep fasting 24 hours before and after drenching, or at least twelve hours before and after, intoxications were relatively much rarer. Slight toxic symptoms were frequently observed, especially loss of appetite and decrease of activity during the first week after treatment, but these symptoms soon vanished. In rare instances the sheep developed diarrhœa, not infrequently bloody, lost all appetite, and died after two or more days. Sometimes they died with symptoms of meteorismus without any diarrhœa. If a 10 per cent. solution of crystalline calcium chloride was given when these symptoms developed the sheep usually recovered. These intoxications, although exceptionally rare (mortality *ca.* 0·007 per cent.) showed a greater tendency to occur where pastures were rich in bushy vegetation (salix and birch). Striking were some cases of acute intoxication observed when sheep were run to the seashore to feed on seaweed shortly after drenching. But on the whole intoxications were so rare that the farmers hardly took any notice of them.

Chabertia Infestation.—When we have taken intestines in autumn for the purpose of investigating worms in them we have found *Chabertia ovina* in such small numbers, usually two or three (*cf.* later) in the colon of a sheep, if any at all, that we did not think this parasite likely to cause much harm.

Last spring one of us was asked to inspect sheep in a district where severe diarrhœa was prevalent among the sheep on some farms and a considerable proportion, particularly yearlings, were lost after one or more weeks of persistent diarrhœa. The rectum

in these sheep was heavily swollen, intensely red, and great numbers of full-grown *Chabertia* were seen adhering to the mucosa, which was sprinkled with pin-point petechiæ from the bites of the worms.

After one drenching with CCl_4 this condition can be cured in all cases, although two drenchings may be necessary sometimes.

This *Chabertia* infestation is apparently very frequent here. We have observed the disease in another place, showing the same clinical picture and changes in rectum and colon, which may be found densely covered with heaps of the worms. The reports we have from farmers and veterinarians in all parts of the country show clearly that *Chabertia* infestations are the cause of the severe diarrhœas which increase in frequency during the latter half of the winter until spring. From these reports we note that they are easily cured by our worm drench.

Further Studies of Intestinal Parasitism in Sheep.—Last autumn one of us (G.G.) made a study of intestinal parasites in sheep from the slaughterhouse, as before. Abomasum and small and large intestines were searched with thorough scrutiny and every worm determined and counted. The following table shows the result :—

Table II.

Ostertagia circumcincta	were found in	80	per cent.	of the sheep.
Nematodirus filicollis	„	40	„	„
Bunostomum trigonocephalum	„	36·4	„	„
Trichocephalus dispar	„	52	„	„
Oesophagostomum venulosum	„	37	„	„
Chabertia ovina	„	46	„	„
Moniezia expansa	„	38	„	

The following table gives a survey of the numbers found of each species :—

Table III.

	Total Number of Sheep in which Worms were found.	*Over 100 Worms were found in*	*From 10 to 100 Worms were found in*	*Less than 10 Worms were found in*	*No Worms were found in*
	Sheep.	Sheep.	Sheep.	Sheep.	Sheep.
Ostertagia circumcincta	92	79	5	8	23
Nematodirus filicollis	47	22	14	11	72
Bunostomum trigonocephalum	43	0	11	32	75
Trichocephalus dispar	63	1	9	53	57
Oesophagostomum venulosum	44	0	1	43	76
Chabertia ovina	55	7	8	40	64

The tables show, in every case, full-grown worms. *Ostertagia* were only to be found in abomasum and nowhere else, except in

two sheep, where they were seen high up in the guts, but very scarce. From the beginning of the observations on September 26th to the end of October great swarms of *Ostertagia* were to be found in every sheep. But after that time a considerable decrease was noted and in several cases the worms were entirely absent. In December only very few *Ostertagia* were found.

Nematodirus filicollis were only found high up, the small intestine. Often many tangles were seen side by side, but sometimes only one here and there.

Bunostomum trigonocephalum were all found high up in the small intestine, sometimes in the same place as *Nematodirus filicollis*, but generally a little lower down. Localisation of worms is very easily perceived owing to the hyperæmic spaces which generally accompany them. Sometimes something resembling remnants of such old bleedings were found, but no worms. In one instance two worms were found in the blind end of the cæcum.

It is interesting to note that *Bunostomum* was found only in 36·4 per cent. in 1935 as against 78 per cent. in 1933. This decrease suggests the efficiency of the worm drench, which, according to our experience, is particularly great against *Bunostomum*. In 1935 we did not see any intestines densely set with bites and devoid of mesenteric fat, as was so frequently observed in 1933.

Trichocephalus dispar and *œsophagostomum venulosum* were only found in the cæcum and where the cæcum and colon meet.

Chabertia ovina seemed to be confined to the large intestine, with but one exception, but as autumn passed a great increase of that worm was apparent, which corresponded to the fact that at this time the majority of sheep are taken into folds and stall fed.

Although the numbers on which Table II is based are rather small it should serve to give some idea of what kinds of worms are the most frequent in the south-western part of Iceland. It may here be stated that the sheep were taken at random from 20 districts within the above-mentioned territory. It is interesting to note that *Hæmonchus contortus* has never been found in this country.

Summary.

Intestinal nematodes in sheep in Iceland have caused severe losses of the stocks in previous years. The most harmful of these worms appears to be *Bunostomum trigonocephalum*, which causes wasting and anæmia, and *Chabertia ovina*, which causes severe diarrhœa with high mortality. Experiments with drugs are reported, carbon tetrachloride giving the best results. After its extensive administration all over the country for two years reports received from about half of the farming population showed that the condition of the sheep had improved definitely or strikingly. Losses from intoxication were very small, 217 sheep being lost of about 300,000 (0·007 per cent.).

A survey is given of the nematodes found in Icelandic sheep.

CYCLOPIA IN THE LAMB.

By Howel V. Hughes,
Department of Veterinary Anatomy, The University, Liverpool.

The subject was a ewe lamb, the progeny of a Welsh Mountain ewe sired by a Kerry Hill ram. It was born dead, and was sent me by the kindness of Mr. D. E. Davies, M.R.C.V.S.

The head was roughly pyramidal in shape, the cranium forming a broad base, and the upper lip tapering away as the apex. The greater part of the face was occupied by the large median eye (Fig. 1). Although the upper and lower eyelids showed no evidence of division into two halves, there being no nasal canthus or membranæ nictitantes, etc., two distinct corneæ

Fig. 1.

Cyclops cyclocephalus in a lamb.

were present, but these were united to one another in the middle line by a portion of the sclerotic, which was a little more transparent than usual. The face was short, the nostrils lying immediately below the eye. The upper lip projected beyond the nostrils

in the form of a short proboscis overhanging the lower lip for some little distance. The nostrils were, however, quite distinct from the proboscis and formed no part of it.

On dissection it was found that the structures of the neck were normal behind the level of the larynx (Fig. 2). The superficial structures of the mandibular space were recognisable, but the deeper structures were indistinct, for the greater part of the space was occupied by a broad plate of bone connecting the horizontal rami to the two auditory bullæ. Only the root of the tongue was developed and this part ended anteriorly in a blunt eminence, the mouth cavity being cut off by the mucous membrane passing up from the floor of the cavity in front of the tongue. A rudimentary soft palate was present, attached in front to the plate of the mandible. Above the soft palate the pharynx led through a narrow slit-like passage into the roof of the mouth. There was a prominent hard palate ending anteriorly in a blunt point. No teeth were erupted. On either side of the palate ran a groove, and here opened the eustachian tubes. The nasal sacs were not apparent, the anterior nares being nothing more than slight depressions in the integument lying below the orbit.

The eye was obviously composed of two elements incompletely connected together. The two levatores palpebræ superioris were separate, as were also the lachrymal glands. The inferior oblique muscles fused in the middle line, and here a large branch of the third cranial nerve entered from either side. The two superior oblique muscles, arising from the back of the orbit, soon blended, and after passing through a pulley in the middle line they joined a transverse band (very similar to the combined inferior oblique muscles) which ran across from one side of the eyeball to the other. The external rectus and the abducens nerve of each eye were well formed. The internal recti were absent. The fourth cranial nerve on each side entered the side of the separate parts of the superior oblique muscles. A single optic nerve ran from a mesially-placed optic foramen to enter the back of the orbit at its centre.

On the exterior of the eyeball, superiorly, there was a faint depression which denoted the line of fusion of the two original elements. Within the eyeball was a median septum, but this was perforated towards the back where the common optic nerve entered, and the vitreous humour of one half mingled with that of the other half. There was a distinct lens and iris for each half of the eyeball.

An examination of the skull bones revealed the fact that the cranial cavity was unusually large (Fig. 3). The parietal bones seemed to be responsible for most of this increase in size, for both showed a prominent bulge into the temporal fossæ considerably reducing the size of these cavities. The interparietal bone was also large. In contrast to the parietals, the frontals were small. The interfrontal suture had entirely disappeared, and in the middle

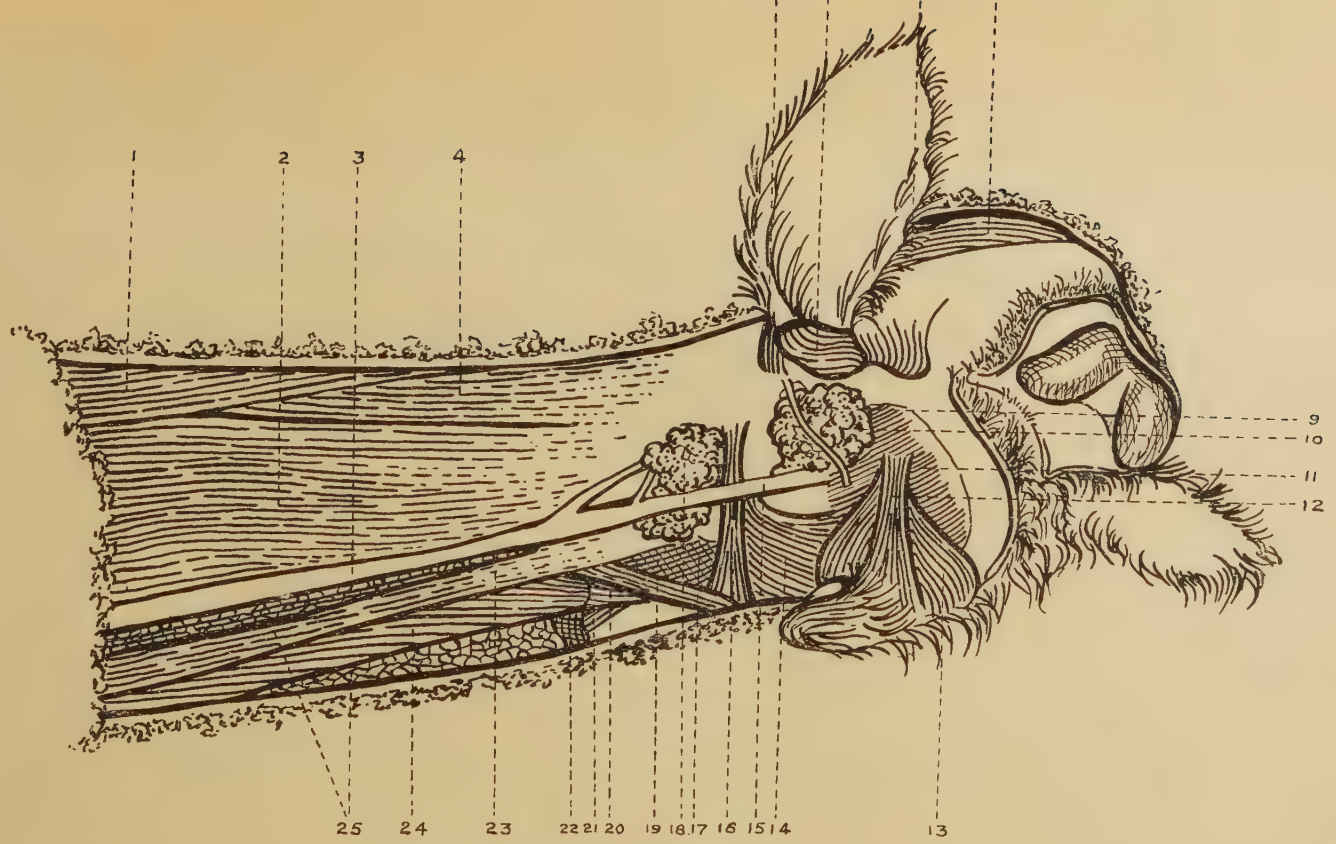


FIG. 2.

Superficial dissection—right side of head and neck.

1, Trapezius m. ; 2, Brachiocephalicus m. ; 3, Jugular v. ; 4, Splenius m. ; 5, Parotido-auricularis m. ; 6, Zygomatico-auricularis m. ; 7, Temporalis m. ; 8, Parieto-auricularis m. ; 9, Facial n. ; 10, Parotid gland ; 11, Masseter m. ; 12, Zygomaticus m. ; 13, Orbicularis and Levator labii superioris m.'s ; 14, Digastricus m. ; 15, Submaxillary v. ; 16, Stylo-hyoid m. ; 17, Pharynx ; 18, Submaxillary lymph gland ; 19, Omo-hyoid m. ; 20, Thyro-hyoid m. ; 21, Crico-thyroid m. ; 22, Cricoid cartilage ; 23, Sterno-mastoid m. ; 24, Sterno-thyro-hyoid m. ; 25, Thymus gland.

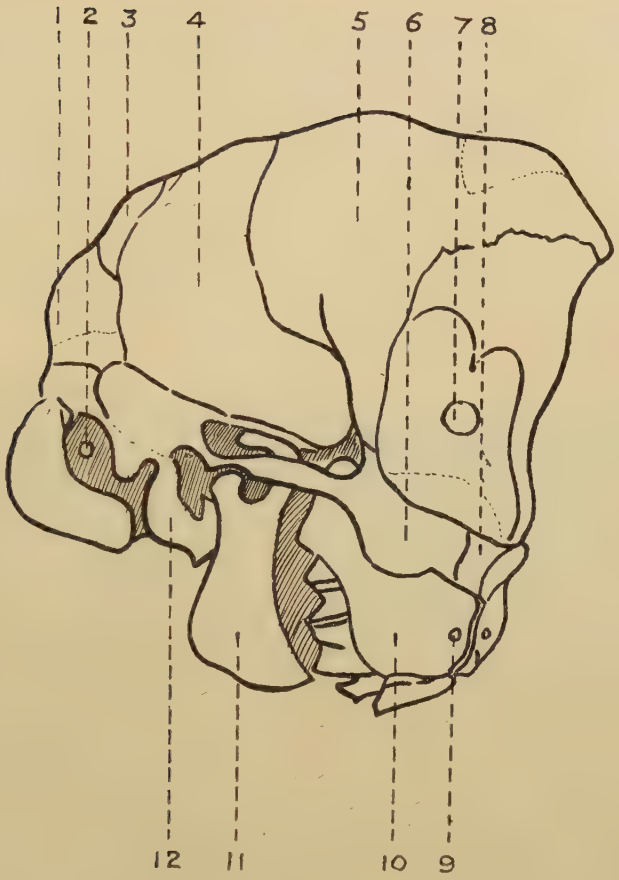


FIG. 3.

Right side of skull.

1, Occipital; 2, Condyloid foramen; 3, Interparietal; 4, Parietal; 5, Frontal; 6, Malar; 7, Optic foramen; 8, Lachrymal; 9, Infraorbital foramen; 10, Superior maxilla; 11, Inferior maxilla; 12, Auditory bulla.

line, behind and above the orbit, there was a large eminence. The single orbital cavity was large, its width being only a little less than the width of the skull itself. It was almost as high as it was broad. The orbital rim was formed very largely by the frontal and malar bones, the lachrymal bones being very small. The last-mentioned bones were fused in the middle line and completed the rim below. The posterior wall of the orbit was formed above by the frontal bones and below by a thin fibrous membrane representing the pre-sphenoid wings. (The ethmoid bone appeared to be undeveloped.) The floor of the cavity was formed largely by the maxillary protuberances.

The face below the orbit was composed of the malar, lachrymal and superior maxillary bones, there being no trace of nasal and premaxillary bones. In each upper jaw four molar teeth were present. The first pair were placed with their long axes lying horizontally across the front of the jaw, their roots being in apposition in the middle line. The auditory bullæ were large and they were connected by a plate of bone to the mandible. It was this plate which was noted earlier as occupying the mandibular space. A narrow aperture between this plate and the basi-sphenoid enabled the pharynx to communicate with the œsophagus. The mandible was short and broad, and showed no traces of incisor or molar teeth. The hyoid bone was practically normal.

FIG. 4.

Right side of brain.

1, Cerebellum; 2, Ant. corpora quadrigemina; 3, Optic thalamus; 4, Stria medullaris; 5, Cerebral hemispheres; 6, Optic nerve; 7, Third nerve; 8, Fourth nerve; 9, Fifth nerve; 10, Seventh nerve; 11, Medulla; 12, Spinal root of Eleventh nerve.

The brain (Fig. 4) occupied only about one-half of the cranial cavity, the remainder of the cavity being filled with fluid. The medulla, pons and cerebellum appeared to be quite normally developed, and they completely filled the posterior fossa of the cranium. In front of this the development of the cerebrum was seen to be arrested, a considerable interval existing between this part of the brain and the overlying cranial wall. The cerebral peduncles and the interpeduncular fossa were normal but the optic

chiasma gave rise to a single large optic nerve. Viewed from above, the most striking feature was the lack of development of the cerebral hemispheres. These formed an irregular mass lying at the anterior end of the brain. The mass was roughly wedge-shaped and was connected to the remainder of the brain at the lower part of its posterior surface. Its anterior surface showed slightly to the left of the middle line an irregular blind depression, but there was no separation of the mass into two hemispheres nor were there any convolutions on its surface. As a result of this defective development the corpora quadrigemina and the optic thalami were uncovered. The olfactory lobes were undeveloped.

Cyclopia has been frequently reported as occurring in domestic animals. In the cases investigated by both Hannover[1] and Taruffi[2] the condition was found to be commonest in pigs, whilst sheep were next most often affected. In recent literature Gladstone and Wakeley[3] have published a detailed account of the condition as occurring in a lamb. Their case, however, was one which, according to St. Hilaire's[4] classification, would be termed Cyclops Rhinocephalus, the nasal chambers being developed into a proboscis-like structure projecting upwards from the frontal region above the orbit. In the present case the nasal chambers were non-existent, and although a proboscis might be said to be present, it was not associated with the nostrils. The condition would come under St. Hilaire's category of Cyclops Cyclocephalus.

Schwalbe and Josephy[5] deal fully with the condition, and outline the theories which have been put forward to explain its origin. They emphasise the fact that the essence of cyclopia lies in an abnormal development of the brain—the optic bulbs arising from this.

A number of workers in experimental embryology have shown that the condition can be produced experimentally. Spemann[6] tied the eggs of triton whilst in the two-celled stage of development, Lewis[7] pierced the eggs of fishes, and Stockard[8] and McClendon[9] subjected the early embryos of fundulus to the action of certain inorganic salts (*e.g.*, salts of magnesium) and other reagents (particularly those having anæsthetic properties such as alcohol, chloroform, chloretone, etc.).

But how these factors produce the condition is not known, and, as Josephy suggests, conditions which have been produced experimentally are not necessarily comparable with conditions occurring naturally. Spemann believes that the condition is the result of a defective development of the central parts of the anterior extremity of the medullary plate. Depending upon the degree of suppression of development in this part of the plate, the eyes may be slightly approximated, or they may be represented by a single eye, or there may even be complete anophthalmia.

In his experimental cases, Spemann noted that the condition occurred by tying " not quite medially but rather obliquely . . . the defective anterior end developing on that half of the embryo which is turned aside from what would virtually be the median plane of the embryo." He argues that the medullary plate is cleft anteriorly by the tying into two unequal parts, the normal head developing from the broad part and the cyclopic head from the narrow part. It is obvious that it is possible for each of the two anterior ends of the original medullary plate to give rise to only those organs the anlagen of which each contained. Thus the so-called teratogenetic end-period of the condition—the latest period in which the cause of the condition could have operated—would at least be as early as the time of the formation of the medullary plate.

More recently Gladstone and Wakeley compared the condition with the class of monsters known as cephalothoracopagus disymmetros, where the head and thorax of two individuals appear fused together. Actually the condition is one of partial duplicitas, and the " double head " shows two faces formed from the opposed halves of each head. The faces are usually unequally developed—that on the side on which the two halves are united at a projecting angle being better developed than that on the opposite side. On this latter side the halves of the face have grown together, but the most median parts (*e.g.*, nose and mouth) of each have failed to develop and the lateral parts have approximated. The eyes have become involved in this approximation. Their median parts have been obliterated and there is formed a single eye composed of what are virtually the lateral parts of the two adjacent eyes of the separate faces.

Cephalothoracopagus disymmetros is a form of duplicitas due to fission of the anterior end of the embryo, but after fission has occurred the two heads have grown forward together. In cyclopia there has also been fission, but it has occurred at a later stage than in cephalothoracopagus disymmetros for the two parts of the anterior end of the embryo on either side of the fissure had already differentiated into the two sides of a single head instead of developing each into a complete head. Spemann believed the fission to occur at the time of the formation of the medullary plate, but Gräper,[10] by means of stereo-cinematographic recordings, has shown that all chick double monsters are due to abnormal streamings of the protoplasm prior to the formation of the primitive streak. Whether or not irregularities of the streaming of the protoplasm are again responsible for the fission at this stage is not known. Nor have we any definite knowledge as to the cause of these irregularities. It would appear, however, that the teratogenetic end-period of cyclopia may be only a little later than that of duplicitas anterior.

The outstanding fact which emerges from a consideration of

the ætiology of cyclopia and allied conditions is the tendency to attribute the abnormality to progressively earlier stages in development. In view of Gräper's hypothesis, having regard also to the fact that abnormalities are known to have resulted from delayed fertilisation (with consequent over-ripeness of the ovum), and bearing in mind McClendon's evidence regarding the effects of toxins on the early embryos, one realises that a very critical stage occurs not only during the early hours succeeding fertilisation but also in connection with the ovum during the period prior to this, a fact which emphasises the great importance of the careful treatment of the dam at this time.

References.

[1]Hannover. *Vidensk. Selsk. Afd. I.*, 9, 1884.
[2]Taruffi. *Storia della Teratologia*, Tomo vi & viii. Bologna, 1891 & 1894.
[3]Gladstone and Wakeley. *Jour. of Anat. & Physiol.*, Vol. liv, p. 196, 1920. London.
[4]St. Hilaire. Histoire générale et particuliere des anomalies de l'organisation chez l'homme et les animaux, 1832-37. Paris.
[5]Schwalbe und Josephy. Sie morphologie der missbildungen des menschen und der tiere. *Teil iii, Lief.* xi, p. 205, 1913. Jena.
[6]Spemann. *Archiv. f. Entwicklungsmech. Bd.*, xvi, 1903.
[7]Lewis. *Anat. Record*, Vol. iii, p. 175, 1909.
[8]Stockard. *Amer. Jour. of Anat.*, p. 369, 1910.
[9]McClendon. *Amer. Jour. of Physiol. Vol.* xix, p. 289, 1912.
[10]Gräper. *Verh. der Anat. Ges. Breslau Anat. Anzeig.*, lxxii, p. 35, 1931.

THE PROTEIN CHANGES IN OX SERUM INDUCED BY HYPERIMMUNISATION AGAINST RINDERPEST VIRUS.

By M. H. French, M.A., DIP. AGRIC. (CANTAB.).

Most of the work done on the chemical changes taking place in the serum of an animal during immunisation and hyperimmunisation has been done in connection with antitoxic sera. The work of Hiss and Atkinson (1901), Ledingham (1907), and Gibson and Banzhaf (1910) had shown that an increase of serum globulin accompanied the production of antitoxins in serum, before any systematic attempt was made to study the changes during the production of potent antiviral sera.

Although Sternberg (1892) had demonstrated the presence of an antiviral body in antivaccinia serum, and Béclère, Chambon and Menard (1899) had shown that this antibody was associated only with the serum globulin, no systematic study of the changes taking place during immunisation or hyperimmunisation against a virus was undertaken until 1914. In that year Hartley showed that the production of potent anti-rinderpest serum by the hyperimmunisation of oxen was accompanied by a marked increase of the total globulins and in particular of the globulin insoluble in saturated NaCl. Hornby (1927) repeated and confirmed Hartley's findings on the protein changes during hyperimmunisation against rinderpest virus.

During the last decade much work has been done on the protein changes accompanying the production of antiviral sera. Thus Maitland and Burbury (1927) concluded that the antibody in the serum of guinea-pigs immunised against foot-and-mouth disease was associated only with the globulins. The findings of Weyer, Park and Banzhaf (1929) and of Morgan and Fairbrother (1930) indicate that the anti-poliomyelitis antibody is associated with both globulins but not with the albumin of the serum. Findlay (1931) and Ledingham, Morgan and Petrie (1931), working with horses hyperimmunised against vaccinia, showed that the antiviral body was distributed through both globulin fractions of the sera but not with the albumin. Laidlaw and Dunkin (1931) concluded that in anti-distemper sera the antibody was confined to the " Felton fraction " on the border-line of the salt precipitation limits for euglobulin and pseudoglobulin.

In the recent work on anti-rinderpest serum Haddow, Sen and Roy (1933) have shown that the total globulin of serum contains more protective properties than the euglobulin fraction.

There is therefore abundant evidence to show that the serum globulin is increased during hyperimmunisation, but the findings of the different workers vary considerably as to the distribution of antibody in the globulin proteins.

The following experiments were undertaken to get further

information on the changes in the serum proteins during hyperimmunisation against rinderpest virus. It was also hoped to get further evidence as to the efficacy of the intrarumenal method of hyperimmunisation.

For the production of anti-rinderpest serum many methods of hyperimmunisation are used, variations in technique and in the route employed being found between almost all the serum institutes. The most common methods are, however, the introduction of a single massive dose of virulent fluid either intramuscularly, intravenously, or subcutaneously. In certain cases the intraperitoneal route is favoured. With the intravenous route anaphylactic shock is of frequent occurrence. With the intramuscular and subcutaneous methods abscess formation is a constant menace, and, in particular with the intramuscular injection of virulent material, much pain and stiffness is caused. Any of the established techniques may be therefore a brutal, and for the ox a painful, procedure. This led Hornby [1927 (1)] to introduce the intrarumenal method of hyperimmunisation into the Tanganyika routine in 1922. This method is very much more humane than any other and, as the serum produced has given satisfactory protection to the local stock, the intrarumenal route has been adopted as the routine procedure for this Territory.

Hartley's (1914) cattle were hyperimmunised by the subcutaneous injection of large doses of defibrinated blood. The most characteristic serum protein change was a large increase of the globulins insoluble in saturated NaCl. Since he had previously shown that the antiviral body of such serum was solely associated with this fraction he suggested that the large increase of this globulin fraction which occurs during " successful immunisation and hyperimmunisation against rinderpest represents, in part at least, an accumulation of rinderpest immune bodies."

As criticisms were levelled against the intrarumenal method of hyperimmunisation, Hornby [1927 (2)] repeated Hartley's work, using the same technique of protein fractionation and estimation. He found that a large increase of the globulins insoluble in saturated NaCl took place during hyperimmunisation, and that this increase was not obtained when normal citrated blood was given intrarumenally. Justification was therefore obtained for the continued use of this more humane method of serum production.

This method has received further adverse criticisms at intervals. It has been claimed that absorption of virus does not take place so completely as with the more common routes. Hall (1929) has shown that, for Nigerian cattle, anti-rinderpest serum prepared by the intrarumenal method was definitely inferior to that obtained from the intramuscular hyperimmunisation. Hall (1933) later attributed the success of intrarumenally produced serum in Tanganyika to the natural resistance of Tanganyika native cattle against rinderpest. More recently Bennett and Evans (1934) report

on a comparative test of antiserum made by the intrarumenal and intramuscular methods in the Sudan. Their results are very clear cut and indicate that in the Sudan the intrarumenal route gives rise to a very inferior antiserum.

Experiment I.—To Determine the Serum Protein Changes during Hyperimmunisation of Oxen by the Intrarumenal Route.

For the purpose of this experiment four immune oxen were chosen at random from amongst the oxen brought in for the routine hyperimmunisation during two successive weeks. The procedure in all four cases was the same. The ox was brought in from grazing and given 2 litres of citrated virulent blood intrarumenally. Ten days later the animal was brought in for the first bleeding, when 1½ litres of blood were drawn. Similar bleedings were made in the two subsequent weeks. The serum protein changes for each ox are shown in the following four tables :—

TABLE I.
Serum Protein changes during Hyperimmunisation. (Ox 2964.)

	5.12.34.	17.12.34.	24.12.34.	31.12.34.
Total N per cent.	1·0931	1·1932	1·1909	1·1914
Non-Protein N per cent.	0·0317	0·0412	0·0412	0·0470
Total Protein N per cent.	1·0614	1·1520	1·1497	1·1444
Euglobulin N per cent.	0·1309	0·1898	0·1866	0·1671
Pseudoglobulin N per cent.	0·4592	0·4959	0·4672	0·4279
Total Globulin N per cent.	0·5901	0·6857	0·6538	0·5950
Albumin N per cent.	0·4713	0·4663	0·4959	0·5494
N per cent. of fraction insoluble in saturated NaCl	0·1733	0·2608	0·2316	0·1900
Euglobulin as per cent. of Protein ...	12·33	16·48	16·23	14·60
Pseudoglobulin as per cent. of Protein	43·27	43·05	40·64	37·40
Albumin as per cent. of Protein ...	44·40	40·47	43·13	48·00
NaCl insoluble fraction as per cent. of Protein	16·33	20·90	20·14	16·60
Total globulin as per cent. of Protein...	55·60	59·53	56·87	52·00

TABLE II.
Serum Protein changes during Hyperimmunisation. (Ox 2399.)

	5.12.34.	17.12.34.	24.12.34.	31.12.34.
Total N per cent.	1·1232	1·0812	1·0507	1·0914
Non-Protein N per cent.	0·0317	0·0412	0·0412	0·0470
Total Protein N per cent.	1·0915	1·0400	1·0095	1·0444
Euglobulin N per cent.	0·1487	0·1750	0·1888	0·1587
Total Globulin N per cent.	0·5414	0·5144	0·5432	0·5392
Pseudoglobulin N per cent.	0·3927	0·3394	0·3544	0·3805
Albumin N per cent.	0·5501	0·5256	0·4663	0·5052
N per cent. in fraction insoluble in saturated NaCl	0·1395	0·2034	0·2424	0·2030
Euglobulin as per cent. of Protein ...	13·62	16·83	18·70	15·20
Pseudoglobulin as per cent. of Protein	35·98	32·63	35·10	36·43
Albumin as per cent. of Protein ...	50·40	50·53	46·20	48·37
NaCl insoluble fraction as per cent. of Protein	12·78	19·56	24·01	19·44
Total Globulin as per cent. of Protein	49·60	49·46	53·80	51·63

The figures obtained show that in only one of these oxen did the increase of total protein occur as reported by Hartley. The euglobulin and the protein insoluble in saturated NaCl showed increases in both the absolute and relative amounts in both oxen, but the increases observed are much smaller than found by Hartley and correspond to those found by Hornby for the ox (No. 1008), which showed the smallest increase in his series of experiments.

TABLE III.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 2966.)

	12.12.34.	24.12.34.	31.12.34.	7.1.35.
Total N per cent.	1·0856	1·1322	1·1932	1·2236
Non-Protein N per cent.	0·0317	0·0412	0·0470	0·0235
Total Protein N per cent.	1·0539	1·0910	1·1462	1·2001
Euglobulin N per cent.	0·0888	0·1964	0·2538	0·2654
Pseudoglobulin N per cent.	0·4499	0·3871	0·3726	0·4345
Total Globulin N per cent.	0·5387	0·5835	0·6264	0·6999
Albumin N per cent.	0·5152	0·5075	0·5198	0·5002
NaCl insoluble fraction N per cent. ...	0·0932	0·3046	0·2928	0·3076
Euglobulin as per cent. of Protein ...	8·43	18·00	22·14	22·11
Pseudoglobulin as per cent. of Protein	42·69	35·48	32·51	36·21
Albumin as per cent. of Protein ...	48·88	46·52	45·35	41·68
NaCl insoluble fraction as per cent. of Protein	8·84	27·92	25·56	25·63
Total Globulin as per cent. of Protein	51·12	62·64	54·65	58·32

TABLE IV.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3027).

	12.12.34.	24.12.34.	31.12.34.	7.1.35.
Total N per cent.	1·0800	1·0812	1·2077	1·2208
Non-Protein N per cent.	0·0317	0·0412	0·0470	0·0235
Total Protein N per cent.	1·0483	1·0400	1·2607	1·1973
Euglobulin N per cent.	0·0749	0·2489	0·2695	0·2342
Pseudoglobulin N per cent.	0·4367	0·3540	0·4874	0·4629
Total Globulin N per cent.	0·5116	0·6029	0·7569	0·6971
Albumin N per cent.	0·5367	0·4371	0·5038	0·5002
N per cent. in fraction insoluble in saturated NaCl	0·0914	0·2725	0·2919	0·2828
Euglobulin as per cent. of Protein ...	7·14	23·93	21·38	19·56
Pseudoglobulin as per cent. of Protein	41·66	34·04	38·66	38·66
Albumin as per cent. of Protein ...	51·20	42·03	39·96	41·78
NaCl insoluble fraction as per cent. of Protein	8·72	26·20	23·15	23·62
Total Globulin as per cent. of Protein	48·80	57·97	60·04	58·22

These two oxen showed changes of serum proteins more similar to those of Hartley.

A rise of total proteins, globulins, euglobulin and globulin insoluble in saturated NaCl was observed. Also the relative amounts of the two latter proteins showed a typical increase, whilst the pseudoglobulin and albumin showed a decrease in their proportional amounts.

The amount of protein insoluble in saturated NaCl was greater in amount than the protein insoluble in 14 per cent. Na_2SO_4 (referred to here as euglobulin). The changes in these two fractions show the same fluctuation tendencies.

The results obtained with these four oxen indicate that the serum protein changes in oxen hyperimmunised intrarumenally are not so constant as was found by Hartley for cattle hyperimmunised subcutaneously.

A further indication that this is so is given by the analyses of samples taken at random from cattle coming in for their first bleeding. Such analyses are given in Table V.

TABLE V.

Analyses of Random Samples of Anti-serum.

	Ox 2865. 1*st* *Bleeding.*	*Ox* 2833. 1*st* *Bleeding.*	*Ox* 2607. 1*st* *Bleeding.*	*Ox* 2983. 1*st* *Bleeding.*	*Ox* 3147. 1*st* *Bleeding.*	*Ox* 3093. 1*st* *Bleeding.*
	21.3.33.	21.8.34.	12.8.34.	22.12.31.	21.8.34.	2.7.35.
Total N per cent. ...	0·9824	1·0303	1·1004	1·0515	1·0313	1·1004
Non-Protein N per cent.	0·0300	0·0265	0·0304	0·0343	0·0318	0·0383
Total Protein N per cent.	0·9524	1·0038	1·0706	1·0172	0·9995	1·0621
Euglobulin N per cent.	0·2170	0·1591	0·2437	0·1899	0·1689	0·1989
Pseudoglobulin N per cent.	0·3704	0·3438	0·3913	0·4022	0·4050	0·3952
Total Globulin N per cent.	0·5874	0·5029	0·6350	0·5921	0·5739	0·5941
Albumin N per cent. ...	0·3650	0·5009	0·4356	0·4251	0·4256	0·4680
Albumin as per cent. of Protein	38·32	49·90	40·69	41·79	42·58	44·06
Pseudoglobulin as per cent of Protein ...	38·89	34·25	36·55	39·54	40·52	37·21
Euglobulin as per cent. of Protein	22·78	15·85	22·76	18·67	16·90	18·73

These figures confirm the results with the previous four oxen that large protein variations can be found in the serum of cattle

TABLE VI.

Analyses of Random Samples of Anti-serum Prepared for Field Use.

	Brew No. 246.	*Brew No.* 253.	*Brew No.* 369.	*Brew No.* 396.
	19.11.31.	8.1.32.	21.11.34.	11.7.35.
Total N per cent.	0·9767	0·9695	0·9824	0·9847
Non-Protein N per cent.	0·0351	0·0287	0·0300	0·0325
Total Protein N per cent.	0·9416	0·9408	0·9524	0·9522
Euglobulin N per cent.	0·1981	0·2101	0·1963	0·2001
Pseudoglobulin N per cent.	0·4348	0·4210	0·3911	0·4121
Total Globulin N per cent.	0·6329	0·6311	0·5874	0·6122
Albumin N per cent.	0·3087	0·3097	0·3650	0·3400
Albumin as per cent. of Protein ...	32·79	32·92	38·32	35·71
Pseudoglobulin as per cent. of Protein	46·16	44·75	41·06	43·28
Euglobulin as per cent. of Protein ...	21·04	22·32	20·61	21·01

hyperimmunised intrarumenally. It is interesting also to compare the figures obtained on four samples of ordinary antiserum as prepared for field use. These field brews are made from the pooled sera of animals being bled for the first, second and third times after receiving their hyperimmunising dose of virulent fluid.

As would be expected, the composition of the pooled product is relatively constant, and indicates that a standardised serum is being prepared in Tanganyika.

Experiment II.—An Attempt to Determine to what Extent the Serum Protein Changes are due to the Virus and not to other Bodies Injected Simultaneously.

In this experiment the serum protein changes are followed in three oxen hyperimmunised against rinderpest virus by means of virulent blood intrarumenally, respectively for the first, ninth and twenty-second times. These changes are compared with those of a control ox herded alongside these three hyperimmunised oxen, and with those for two other oxen given normal citrated blood intrarumenally instead of virulent blood. A seventh ox was given, intrarumenally, colostrum diluted with normal saline so as to make its total protein content equal to that of normal blood.

TABLE VII.

Serum Protein Analyses of a Control Ox for a Period of Six Weeks. (Ox 2845.)

	17.7.34.	30.7.34.	13.8.34.	27.8.34.
Total N per cent.	1·0165	1·0067	1·0021	1·0102
Non-Protein N per cent.	0·0382	0·0403	0·0351	0·0343
Total Protein N per cent.	0·9783	0·9664	0·9670	0·9759
Fibrinogen N per cent.	0·0213	0·0259	0·0228	0·0239
Euglobulin N per cent.	0·0668	0·0632	0·0661	0·0652
Pseudoglobulin N per cent.	0·4006	0·3814	0·3887	0·4094
Total Globulin N per cent.	0·4674	0·4446	0·4548	0·4746
Albumin N per cent.	0·4896	0·4959	0·4894	0·4774
Albumin as per cent. of Protein ...	50·05	51·31	50·61	48·91
Fibrinogen as per cent. of Protein ...	2·18	2·68	2·36	2·45
Euglobulin as per cent. of Protein ...	6·83	6·54	6·83	6·68
Pseudoglobulin as per cent. of Protein	40·94	39·47	40·20	41·96

Control Ox 2845.—This ox had been immunised some months previous to this experiment and so the serum analyses represent the normal values for an ox subjected to the grazing and management conditions of the experimental animals. The total protein content varied slightly through the experiment but the relative proportions of the different protein fractions remained very constant for the whole six weeks.

Oxen S and 2900.—Two oxen were given 2 litres of citrated normal blood intrarumenally in the same manner as an ox being hyperimmunised. One ox was a susceptible animal, whilst the other was an immune beast. The serum protein changes in these two oxen are given in Tables VIII and IX.

TABLE VIII.

SERUM PROTEIN CHANGES IN A SUSCEPTIBLE OX GIVEN NORMAL BLOOD INTRARUMENALLY. (OX S.)

	17.7.34.	24.7.34.	31.7.34.	8.8.34.	15.8.34.	22.8.34.	28.8.34.
Total N per cent.	1·0231	1·1122	1·1368	1·0870	1·0669	1·1358	1·0399
Non-Protein N per cent.	0·0403	0·0403	0·0445	0·0435	0·0388	0·0388	0·0291
Total Protein N per cent.	0·9828	1·0719	1·0723	1·0435	1·0281	1·0970	1·0108
Fibrinogen N per cent.	0·0249	0·0251	0·0258	0·0228	0·0301	0·0351	0·0265
Euglobulin N per cent.	0·0806	0·1695	0·1434	0·1166	0·1088	0·0947	0·0747
Pseudoglobulin N per cent.	0·3756	0·3800	0·4157	0·4076	0·4093	0·4827	0·4548
Total Globulin N per cent.	0·4562	0·5495	0·5591	0·5242	0·5181	0·5774	0·5195
Albumin N per cent.	0·5017	0·4973	0·4874	0·4965	0·4799	0·4845	0·4548
Albumin as per cent. of Protein	51·05	46·40	45·46	47·58	46·68	44·17	45·00
Fibrinogen as per cent. of Protein	2·53	2·34	2·41	2·19	2·93	3·20	2·62
Euglobulin as per cent. of Protein	8·20	15·81	13·37	11·17	10·58	8·63	7·39
Pseudoglobulin as per cent. of Protein ...	38·22	35·45	38·76	39·06	39·81	44·00	44·99

TABLE IX.

SERUM PROTEIN CHANGES IN AN IMMUNE OX GIVEN NORMAL BLOOD INTRARUMENALLY. (OX 2900.)

	17.7.34.	24.7.34.	31.7.34.	8.8.34.	15.8.34.	22.8.34.	28.8.34.
Total N per cent.	1·0160	1·0654	1·1918	1·1813	1·0300	1·0790	1·0855
Non-Protein N per cent.	0·0520	0·0504	0·0403	0·0520	0·0423	0·0388	0·0265
Total Protein N per cent.	0·9640	1·0150	1·1515	1·1293	0·9877	1·0402	1·0590
Fibrinogen N per cent.	0·0395	0·0395	0·0365	0·0399	0·0389	0·0308	0·0332
Euglobulin N per cent.	0·0630	0·0817	0·0916	0·0782	0·0794	0·0702	0·0702
Pseudoglobulin N per cent.	0·4324	0·4466	0·5318	0·4911	0·4480	0·4283	0·3999
Total Globulin N per cent.	0·4954	0·5283	0·6234	0·5693	0·5274	0·4985	0·4701
Albumin N per cent.	0·4291	0·4472	0·4916	0·5201	0·4214	0·5109	0·5557
Albumin as per cent. of Protein	44·50	44·06	42·69	46·06	42·67	49·12	52·47
Fibrinogen as per cent. of Protein	4·10	3·89	3·17	3·53	3·94	2·96	3·13
Euglobulin as per cent. of Protein	6·54	8·05	7·95	6·92	8·03	6·75	6·63
Pseudoglobulin as per cent. of Protein ...	44·86	44·00	46·19	43·49	45·36	41·17	37·77

The figures for the serum proteins of Ox 2900 indicate that the injection of normal blood had no effect on the serum proteins. With Ox S, however, a steady fall of albumin took place over the six weeks' investigation, whilst a steady rise of pseudoglobulin took place. The euglobulin fraction showed a temporary increase and then a return to normal values by the end of the fifth week. The relative changes in the various fractions were a temporary increase of the percentage amount of euglobulin, a steady increase of the pseudoglobulin proportions with a fall in the albumin content.

Ox 3091.—Colostrum contains a globulin closely related to serum globulin and which is readily absorbed from the alimentary tract. After dilution to the protein level of normal blood, 2 litres were injected into the rumen of an immune ox, and the serum protein changes followed for a period of six weeks.

The relative changes in the serum proteins of this ox are an increase of the euglobulin proportions with a return to normal by the fifth week. After a temporary rise the pseudoglobulin proportions fell steadily, whilst the reverse took place with the albumin percentages.

Oxen 2842, 3609 *and* 2074.—These three oxen hyperimmunised with virulent blood (2 litres) were subjected to exactly the same conditions as the above four oxen. Their serum protein variations are given in Tables XI, XII and XIII.

The hope that it would be possible to compare the changes in a newly-hyperimmunised ox with an ox that had been hyperimmunised many times before was not realised because it is doubtful if two of the above animals were successfully hyperimmunised.

The serum protein changes for Oxen 2842 and 2074 are similar to those found for Oxen 2964 and 2399 of Experiment I, and are of the same nature and order as those found for oxen given normal blood or colostrum intrarumenally.

The changes found in the serum of Ox 3609 resemble those for Oxen 2966 and 3027 of Experiment I. They are also similar to those found by Hartley for animals successfully hyperimmunised by the subcutaneous route, though the relative increase of euglobulin was not so great.

Experiment III.—To Compare the Serum Protein Changes in Oxen Hyperimmunised by Different Methods.

One of the advantages of the intrarumenal route for hyperimmunisation is that other organs rich in virus can be used as well as blood. Aqueous or saline extracts of pulped organs cannot be given in large amounts intravenously and will cause much trouble from abscesses when given under the skin or intramuscularly. Such extracts can be given with impunity intrarumenally, and

TABLE X.

SERUM PROTEIN CHANGES AFTER THE INTRARUMENAL INJECTION OF COLOSTRUM. (OX 3091.)

	17.7.34.	24.7.34.	31.7.34.	7.8.34.	14.8.34.	21.8.34.	28.8.34.
Total N per cent.	1·0230	1·1169	1·1158	1·1208	1·0300	1·0956	1·1187
Non-Protein N per cent.	0·0338	0·0403	0·0323	0·0435	0·0291	0·0294	0·0388
Total Protein N per cent.	0·9892	1·0766	1·0835	1·0773	1·0009	1·0662	1·0799
Fibrinogen N per cent.	0·0296	0·0510	0·0304	0·0310	0·0298	0·0391	0·0441
Euglobulin N per cent.	0·0641	0·1207	0·1573	0·1304	0·1021	0·1048	0·0961
Pseudoglobulin N per cent.	0·4171	0·4736	0·4142	0·3435	0·3618	0·3752	0·3484
Total Globulin N per cent.	0·4812	0·5943	0·4715	0·4739	0·4639	0·4800	0·4445
Albumin N per cent.	0·4784	0·4313	0·4816	0·5724	0·5072	0·5471	0·5913
Albumin as per cent of Protein	47·55	40·06	44·45	53·13	50·67	51·31	54·76
Fibrinogen as per cent. of Protein	3·00	4·74	2·80	2·88	2·98	3·67	4·08
Euglobulin as per cent. of Protein	6·48	11·21	14·52	12·10	11·20	9·83	8·90
Pseudoglobulin as per cent. of Protein ...	42·17	43·99	38·23	31·89	36·15	35·19	32·26

TABLE XI.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 2842.) (FIRST HYPERIMMUNISATION.)

	18.7.34.	25.7.35.	1.8.34.	8.8.34.	15.8.34.	22.8.34.	28.8.34.
Total N per cent.	1·0163	1·0738	1·0548	1·0300	0·9898	1·0864	1·0286
Non-Protein N per cent.	0·0403	0·0432	0·0400	0·0435	0·0291	0·0294	0·0265
Total Protein N per cent.	0·9760	1·0306	1·0148	0·9865	0·9607	1·0570	1·0021
Fibrinogen N per cent.	0·0286	0·0282	0·0291	0·0289	0·0285	0·0285	0·0281
Euglobulin N per cent.	0·0701	0·1075	0·1382	0·1024	0·0847	0·0847	0·0801
Pseudoglobulin N per cent.	0·3894	0·4375	0·3719	0·3892	0·3995	0·4823	0·4291
Total Globulin N per cent.	0·4595	0·5450	0·5101	0·4916	0·4842	0·5670	0·5092
Albumin N per cent.	0·4879	0·4574	0·4756	0·4660	0·4480	0·4615	0·4648
Albumin as per cent. of Protein	50·00	44·38	46·87	47·24	46·63	43·64	46·38
Fibrinogen as per cent. of Protein	2·92	2·74	2·87	2·93	2·97	2·69	2·80
Euglobulin as per cent. of Protein	7·18	10·43	13·62	10·38	8·82	8·05	7·80
Pseudoglobulin as per cent. of Protein ...	39·90	42·48	36·64	39·45	41·58	45·62	42·82

TABLE XII.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3609.) (NINTH HYPERIMMUNISATION.)

	17.7.34.	30.7.34.	6.8.34.	13.8.34.	20.8.34.	27.8.34.
Total N per cent.	1·0641	1·1639	1·1876	1·0300	0·9467	1·0402
Non-Protein N per cent.	0·0333	0·0403	0·0520	0·0391	0·0291	0·0388
Total Protein N per cent.	1·0308	1·1236	1·1356	0·9909	0·9176	1·0014
Fibrinogen N per cent.	0·0243	0·0286	0·0462	0·0310	0·0255	0·0308
Euglobulin N per cent.	0·1222	0·2221	0·2998	0·1611	0·1303	0·1045
Pseudoglobulin N per cent.	0·4450	0·4488	0·3901	0·3552	0·3967	0·4020
Total Globulin N per cent.	0·5672	0·6709	0·5999	0·5163	0·5270	0·5065
Albumin N per cent.	0·4393	0·4241	0·4895	0·4436	0·3651	0·4641
Albumin as per cent. of Protein	42·62	37·74	43·11	44·77	39·79	46·34
Fibrinogen as per cent. of Protein	2·36	2·54	4·07	3·13	2·78	3·08
Euglobulin as per cent. of Protein	11·85	19·77	18·48	16·26	14·20	10·44
Pseudoglobulin as per cent. of Protein	43·17	39·95	34·34	35·84	43·23	40·14

TABLE XIII.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 2074.) (TWENTY-SECOND HYPERIMMUNISATION.)

	18.7.34.	25.7.34.	1.8.34.	8.8.34.	15.8.34.	20.8.34.	27.8.34.
Total N per cent.	1·0174	1·1313	1·1292	1·1458	1·0485	1·0300	1·0514
Non-Protein N per cent.	0·0403	0·0403	0·0400	0·0435	0·0388	0·0391	0·0388
Total Protein N per cent.	1·0371	1·0910	1·0892	1·1023	1·0097	0·9909	1·0126
Fibrinogen N per cent.	0·0281	0·0279	0·0264	0·0252	0·0271	0·0271	0·0341
Euglobulin N per cent.	0·0579	0·1078	0·1312	0·1438	0·1233	0·0913	0·0924
Pseudoglobulin N per cent.	0·4588	0·4394	0·4077	0·4320	0·3752	0·4035	0·4154
Total Globulin N per cent.	0·5167	0·5472	0·5489	0·5758	0·4985	0·4948	0·5078
Albumin N per cent.	0·4923	0·5159	0·5239	0·5013	0·4841	0·4690	0·4707
Albumin as per cent. of Protein	47·47	47·29	48·10	45·48	47·95	47·33	46·48
Fibrinogen as per cent. of Protein	2·71	2·56	2·42	2·29	2·68	2·74	3·37
Euglobulin as per cent. of Protein	5·58	9·88	12·05	13·04	12·21	9·21	9·13
Pseudoglobulin as per cent. of Protein ...	44·24	40·27	37·43	39·19	37·16	40·72	41·02

thereby effect a great reduction of the costs of serum production.

In this institution the liver, spleen, kidneys and heart together with several of the larger lymphatic glands of the virus giver are minced finely and mixed with an equal weight of water. After standing from one to two hours, the supernatant fluid is drained off and injected at the rate of 2 litres per beast intrarumenally. Such an extract will be referred to below as " maina."

In this experiment four oxen were hyperimmunised intrarumenally with virulent blood, four others with virulent maina, whilst three oxen were hyperimmunised by the intramuscular route with virulent blood.

The serum protein changes in the four oxen given virulent blood intrarumenally are shown in the next four tables.

TABLE XIV.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3929). (2 LITRES VIRULENT BLOOD INTRARUMENALLY).

	22.5.35.	4.6.35.	17.6.35.	1.7.35.
Total N per cent.	1·1868	1·2152	1·2698	1·0570
Non-Protein N per cent.	0·0353	0·0352	0·0367	0·0359
Total Protein N per cent.	1·1515	1·1800	1·2331	1·0211
Euglobulin N per cent.	0·1248	0·1569	0·1871	0·1557
Pseudoglobulin N per cent.	0·4833	0·4114	0·5387	0·3938
Total Globulin N per cent.	0·6081	0·6483	0·7258	0·5495
Albumin N per cent.	0·5434	0·5317	0·5073	0·4716
N per cent. of Protein insoluble in saturated NaCl	0·1463	0·1784	0·1904	0·1603
Albumin as per cent. of Protein ...	47·19	45·06	41·14	46·18
Pseudoglobulin as per cent. of Protein	41·97	41·64	43·69	38·57
Euglobulin as per cent. of Protein ...	10·84	13·30	15·17	15·25
Saturated NaCl insoluble Protein as per cent. of total Protein	12·70	15·12	15·44	15·70

TABLE XV.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3933). (2 LITRES VIRULENT BLOOD INTRARUMENALLY).

	22.5.35.	4.6.35.	17.6.35.	1.7.35.
Total N per cent.	0·9993	1·0500	0·9634	0·9464
Non-Protein N per cent.	0·0361	0·0353	0·0353	0·0334
Total Protein N per cent.	0·9632	1·0147	0·9281	0·9130
Euglobulin N per cent.	0·1049	0·1487	0·1417	0·0876
Pseudoglobulin N per cent.	0·3845	0·4344	0·3369	0·3528
Total Globulin N per cent.	0·4894	0·5831	0·4843	0·4404
Albumin N per cent.	0·4738	0·4316	0·4438	0·4726
N per cent. of Protein insoluble in saturated NaCl	0·1502	0·1828	0·1684	0·1252
Albumin as per cent. of Protein ...	49·19	42·53	47·81	51·77
Pseudoglobulin as per cent. of Protein	39·92	42·81	36·30	38·64
Euglobulin as per cent. of Protein ...	10·89	14·65	15·88	9·59
Saturated NaCl insoluble Protein as per cent. of total Protein	13·52	17·92	18·12	13·71

TABLE XVI.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3977.)
(2 LITRES VIRULENT BLOOD INTRARUMENALLY.)

	29.5.35.	10.6.35.	17.6.35.	24.6.35.	1.7.35.	8.7.35.
Total N per cent. ...	1·1480	1·2194	1·1046	1·1232	1·0990	1·1235
Non-Protein N per cent.	0·0323	0·0400	0·0349	0·0352	0·0400	0·0384
Total Protein N per cent.	1·1157	1·1794	1·0697	1·0880	1·0590	1·0851
Euglobulin N per cent.	0·1574	0·1841	0·2037	0·1761	0·1265	0·1254
Pseudoglobulin N per cent.	0·4384	0·5319	0·4705	0·4867	0·5203	0·4567
Total Globulin N per cent.	0·5958	0·7160	0·6742	0·6628	0·6468	0·5821
Albumin N per cent. ...	0·5199	0·4634	0·3955	0·4252	0·4122	0·5030
N per cent. of Protein insoluble in saturated NaCl	0·1656	0·2618	0·2254	0·1954	0·1306	0·1305
Albumin as per cent. of Protein	46·60	39·29	36·97	39·08	38·92	46·36
Pseudoglobulin as per cent. of Protein ...	39·29	45·10	43·98	44·73	49·13	42·08
Euglobulin as per cent. of Protein	14·11	15·61	19·04	16·19	11·95	11·56
Saturated NaCl insoluble Protein as per cent. of total Protein	14·84	22·19	21·07	17·96	12·33	12·03

TABLE XVII.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3980.)
(2 LITRES VIRULENT BLOOD INTRARUMENALLY.)

	29.5.35.	10.6.35.	17.6.35.	24.6.35.	1.7.35.	8.7.35.
Total N per cent. ...	1·1634	1·2152	1·1046	1·1872	1·2264	1·2810
Non-Protein N per cent.	0·0323	0·0411	0·0349	0·0359	0·0414	0·0434
Total Protein N per cent.	1·1311	1·1741	1·0697	1·1513	1·1850	1·2436
Euglobulin N per cent.	0·1504	0·2799	0·2845	0·2671	0·1992	0·1758
Pseudoglobulin N per cent.	0·3959	0·3710	0·4344	0·4436	0·5212	0·5084
Total Globulin N per cent.	0·5463	0·6509	0·7189	0·7107	0·7204	0·6842
Albumin N per cent. ...	0·5848	0·5232	0·3508	0·4406	0·4646	0·5594
N per cent. of Protein insoluble in saturated NaCl	0·1642	0·3036	0·3402	0·2568	0·2257	0·1885
Albumin as per cent. of Protein	51·70	44·56	32·80	38·27	39·21	44·98
Pseudoglobulin as per cent. of Protein ...	35·00	31·60	40·61	38·53	43·98	40·87
Euglobulin as per cent. of Protein	13·30	23·84	26·59	23·20	16·81	14·14
Saturated NaCl insoluble Protein as per cent. of Protein ...	14·51	25·86	31·80	22·30	19·04	15·16

The significant facts about these figures are that in only one ox (No. 3980) did the relative amounts of euglobulin and of globulin insoluble in saturated NaCl show increases of the same order as found by Hartley. Ox 3977 showed an increase of the pseudoglobulin fraction as well as a moderate increase in the proportions

of euglobulin. With Oxen 3929 and 3933 the euglobulin proportional increase was small and no larger than that obtained after the injection of normal blood or colostrum. The changes in the protein insoluble in saturated NaCl were also much smaller than the changes found by Hartley.

For the four oxen given maina intrarumenally, the maina was prepared from the same animals that yielded the blood used for both the intrarumenal and intramuscular injections. The serum protein changes in these four animals are given below.

TABLE XVIII.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3932.)
(2 LITRES OF MAINA INTRARUMENALLY.)

	22.5.35.	4.6.35.	17.6.35.	1.7.35.
Total N per cent.	1·1189	1·1494	1·1788	1·1858
Non-Protein N per cent.	0·0359	0·0369	0·0353	0·0363
Total Protein N per cent.	1·0830	1·1125	1·1435	1·1495
Euglobulin N per cent.	0·1310	0·2724	0·2166	0·1586
Pseudoglobulin N per cent.	0·4210	0·3857	0·4588	0·4304
Total Globulin N per cent.	0·5320	0·6581	0·6754	0·5890
Albumin N per cent.	0·5310	0·4544	0·4681	0·5605
N per cent. of Protein insoluble in saturated NaCl	—	0·2870	0·2492	0·1801
Albumin as per cent. of Protein ...	49·03	40·84	40·94	48·76
Pseudoglobulin as per cent. of Protein	38·87	34·67	40·12	37·44
Euglobulin as per cent. of Protein ...	12·10	24·49	18·94	13·80
NaCl insoluble Protein as per cent. of total Protein	13·85	25·79	21·80	15·67

TABLE XIX.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3935.)
(2 LITRES OF MAINA INTRARUMENALLY.)

	29.5.35.	10.6.35.	17.6.35.	24.6.35.	1.7.35.	8.7.35.
Total N per cent. ...	1·1158	1·1984	1·1788	1·1458	1·1564	1·2950
Non-Protein N per cent.	0·0352	0·0335	0·0349	0·0362	0·0385	0·0410
Total Protein N per cent.	1·0806	1·1649	1·1439	1·1096	1·1179	1·2540
Euglobulin N per cent.	0·0602	0·1631	0·2653	0·2054	0·2348	0·1826
Pseudoglobulin N per cent.	0·4303	0·5319	0·4831	0·4152	0·3694	0·5333
Total Globulin N per cent.	0·4906	0·6950	0·7484	0·6206	0·6042	0·7159
Albumin N per cent. ...	0·5900	0·4699	0·3955	0·4890	0·5137	0·5381
N per cent. of Protein insoluble in saturated NaCl	0·0798	0·1960	0·2810	0·2412	0·2410	0·2072
Albumin as per cent. of Protein	54·60	40·34	34·57	44·07	45·95	42·91
Pseudoglobulin as per cent. of Protein ...	39·83	45·66	42·23	37·42	33·04	42·53
Euglobulin as per cent. of Protein	5·57	14·00	23·93	18·51	21·00	14·56
NaCl insoluble Protein as per cent. of total Protein	7·39	16·83	24·56	21·73	21·56	16·52

TABLE XX.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3974.)
(2 LITRES OF MAINA INTRARUMENALLY.)

	29.5.35.	10.6.35.	17.6.35.	24.6.35.	1.7.35.	8.7.35.
Total N per cent. ...	1·2600	1·1956	1·0458	1·0458	1·0545	1·0934
Non-Protein N per cent.	0·0323	0·0400	0·0384	0·0368	0·0349	0·0334
Total Protein N per cent.	1·2277	1·1556	1·0074	1·0090	1·0196	1·0600
Euglobulin N per cent.	0·1638	0·1630	0·1810	0·1744	0·1654	0·1758
Pseudoglobulin N per cent.	0·4386	0·5292	0·4126	0·3705	0·4032	0·4223
Total Globulin N per cent.	0·6024	0·6922	0·5936	0·5449	0·5686	0·5981
Albumin N per cent....	0·6253	0·4634	0·4138	0·4641	0·4510	0·4619
N per cent. of Protein insoluble in saturated NaCl	0·1640	0·2380	0·2170	0·2013	0·1856	0·2085
Albumin as per cent. of Protein	53·38	40·10	41·07	46·00	42·33	39·82
Pseudoglobulin as per cent. of Protein ...	35·97	45·79	40·96	36·72	39·55	43·58
Euglobulin as per cent. of Protein	13·34	14·11	17·97	17·28	16·22	16·59
Saturated NaCl insoluble protein as per cent. of total Protein	13·36	20·60	21·54	19·95	18·20	19·67

TABLE XXI.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3976.)
(2 LITRES OF MAINA INTRARUMENALLY.)

	22.5.35.	4.6.35.	17.6.35.	1.7.35.
Total N per cent.	1·1218	1·1362	1·2110	1·2010
Non-Protein N per cent.	0·0333	0·0369	0·0353	0·0349
Total Protein N per cent.	1·0885	1·0993	1·1757	1·1671
Euglobulin N per cent.	1·1412	0·2598	0·2001	0·1601
Pseudoglobulin N per cent.... ...	0·4050	0·4345	0·5073	0·4530
Total Globulin N per cent.	0·5462	0·6943	0·7074	0·6131
Albumin N per cent.	0·5423	0·4050	0·4683	0·5540
N per cent. of Protein insoluble in saturated NaCl	0·1599	0·2896	0·2341	0·1821
Albumin as per cent. of Protein ...	49·82	36·84	39·83	47·47
Pseudoglobulin as per cent. of Protein	37·21	39·52	43·15	38·81
Euglobulin as per cent. of Protein ...	12·97	23·64	17·02	13·72
NaCl insoluble protein as per cent. of total Protein	14·69	26·34	19·92	15·60

The changes in Oxen 3932 and 3976 showed a marked increase of the relative amounts of euglobulin and of protein insoluble in saturated NaCl. Almost as great an increase was found for the changes of euglobulin in the serum of Ox 3935 though the maximum was reached somewhat later than with the two previous oxen. Also for Ox 3935 the pseudoglobulin proportions showed a temporary increase, falling again as euglobulin proportions reached their maximal value.

Ox 3974 showed only a moderate increase of the proportions of euglobulin and protein insoluble in saturated NaCl, though a rise also occurred in the relative amount of pseudoglobulin. The

changes for this ox are, however, greater than for an animal given normal blood.

As was stated before, the three oxen given virulent blood intramuscularly were hyperimmunised with the same pooled virulent blood as that used for the previous four intrarumenally hyperimmunised oxen. The serum protein changes are as shown in the following tables.

TABLE XXII.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3120.)
(1 LITRE VIRULENT BLOOD INTO NECK MUSCLES.)

	29.5.35.	11.6.35.	17.6.35.	24.6.35.	1.7.35.	8.7.35.
Total N per cent. ...	1·1634	1·2376	1·1994	1·1046	1·2068	1·1298
Non-Protein N per cent.	0·0323	0·0400	0·0349	0·0353	0·0375	0·0333
Total Protein N per cent.	1·1311	1·1976	1·1645	1·0693	1·1693	1·0965
Euglobulin N per cent.	0·1281	0·2997	0·2859	0·2245	0·1918	0·2122
Pseudoglobulin N per cent.	0·4182	0·4791	0·4831	0·4361	0·4628	0·4223
Total Globulin N per cent.	0·5463	0·7788	0·7690	0·6606	0·6546	0·6345
Albumin N per cent....	0·5848	0·4188	0·3955	0·4087	0·5147	0·4620
Protein insoluble saturated NaCl N per cent.	0·1320	0·3258	0·3391	0·2891	0·2613	0·2391
Albumin as per cent. of Protein	51·70	34·97	33·96	38·22	44·02	42·14
Pseudoglobulin as per cent. of Protein ...	36·97	40·00	41·49	40·78	39·58	38·51
Euglobulin as per cent. of Protein	11·33	25·03	24·55	21·00	16·40	19·35
Protein insoluble in saturated NaCl as per cent. of Protein ...	11·67	27·20	29·12	26·10	22·35	21·80

TABLE XXIII.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (OX 3715.)
(1 LITRE VIRULENT BLOOD INTO NECK MUSCLES.)

	29.5.35.	10.6.35.	17.6.35.	24.6.35.	1.7.35.	8.7.35.
Total N per cent. ...	1·1840	1·1816	1·1872	1·0458	1·1284	1·2380
Non-Protein N per cent.	0·0323	0·0235	0·0353	0·0338	0·0351	0·0362
Total Protein N per cent.	1·1517	1·1581	1·1519	1·0120	1·0933	1·2018
Euglobulin N per cent.	0·1284	0·2437	0·2493	0·2144	0·2274	0·2252
Pseudoglobulin N per cent.	0·4304	0·4345	0·3370	0·2549	0·3935	0·3953
Total Globulin N per cent.	0·5588	0·6782	0·5863	0·4693	0·6209	0·6205
Albumin N per cent. ...	0·5929	0·4799	0·5656	0·5427	0·4724	0·5813
N per cent. of Protein insoluble in saturated NaCl	0·1956	0·2688	0·2912	0·2182	0·2317	0·2821
Albumin as per cent. of Protein	51·48	41·44	49·10	53·63	43·20	48·37
Pseudoglobulin as per cent. of Protein ...	37·37	37·52	29·26	25·19	36·00	32·89
Euglobulin as per cent. of Protein	11·15	21·04	21·64	21·18	20·80	18·74
Saturated NaCl insoluble Protein as per cent. of total Protein	16·98	23·10	25·28	21·56	21·19	22·79

TABLE XXIV.

SERUM PROTEIN CHANGES DURING HYPERIMMUNISATION. (Ox 3973.)
(1 LITRE VIRULENT BLOOD INTO NECK MUSCLES.)

	22.5.35.	4.6.35.	17.6.35.	1.7.35.
Total N per cent.	1·1689	1·1900	1·2040	1·1654
Non-Protein N per cent.	0·0341	0·0367	0·0353	0·0381
Total Protein N per cent.	1·1348	1·1533	1·1687	1·1273
Euglobulin N per cent.	0·1279	0·3130	0·2931	0·2010
Pseudoglobulin N per cent.	0·4202	0·4660	0·5049	0·4999
Total globulin N per cent.	0·5481	0·7799	0·7980	0·7009
Albumin N per cent.	0·5867	0·3734	0·3707	0·4264
N per cent. of Protein insoluble in saturated NaCl	0·1581	0·3348	0·3231	0·2301
Albumin as per cent. of Protein ...	51·70	32·38	31·72	37·83
Pseudoglobulin as per cent. of Protein	37·03	40·48	43·20	44·34
Euglobulin as per cent. of Protein Saturated NaCl insoluble Protein as per cent. of total Protein	11·27 13·93	27·14 29·03	25·08 27·65	17·83 20·41

TABLE XXV.

THE NATURE OF THE GLOBULIN INCREASES DURING HYPERIMMUNISATION.

Method of Hyperimmunisation.	*No. of Cattle.*	*Globulin Fraction Increases.*		
		Euglobulin.	*Globulin insoluble in saturated NaCl.*	*Pseudoglobulin.*
Intramuscularly with virulent blood	3	Marked increase in all cases	Marked increase in all cases	Two cases showed a slight increase.
Intrarumenally with extract of pulped virus-containing organs	3	Marked increase	Marked increase	Slight increase.
	1	Moderate increase	Good increase	Slight increase.
Intrarumenally with virulent blood	2	Marked increase	Marked increase	Slight decrease.
	1	Marked increase	Marked increase	Slight increase.
	2	Moderate increase	Good increase	Slight decrease.
	1	Moderate increase	N.D.	Slight decrease.
	1	Moderate increase	Good increase	Slight increase.
	2	Not significant	Not significant	Not significant.
	2	Not significant	N.D.	Not significant.

These cases differ considerably from those of the four animals hyperimmunised intrarumenally. Here, all three oxen showed a marked increase of euglobulin and globulin insoluble in saturated NaCl. Also two oxen showed a slight increase in the proportions of pseudoglobulin. The changes in the euglobulin and NaCl insoluble proteins are of the same order and magnitude as those found by Hartley for animals successfully hyperimmunised subcutaneously.

The cases examined in this investigation can therefore be collected into groups as has been done in Table XXV, according to the nature of the globulin changes taking place during hyperimmunisation.

Thus, whilst all the animals given virulent blood intramuscularly gave marked increases in both the euglobulin and saturated NaCl insoluble globulin, this occurred in only three out of eleven animals given virulent blood intrarumenally, and three out of four oxen given the extract of pulped virulent organs intrarumenally. Four out of eleven oxen given virulent blood intrarumenally gave changes no greater than those produced by normal citrated blood. Relative terms were used in Table XXV to compare the changes. The euglobulin increase was " not significant," " moderate " or " marked " according as the euglobulin proportional value increased to 15 per cent., from 15 to 20 per cent., and over 20 per cent. respectively of the total protein content. Similarly, for the globulin insoluble in saturated NaCl the proportional increases were grouped, up to 18 per cent., 18 to 22 per cent., and over 22 per cent. respectively into, " not significant," " good " and " marked."

Discussion.

From the foregoing experiments it appears that the greatest changes in the serum proteins during hyperimmunisation are large increases of the relative proportions of euglobulin and of globulin insoluble in saturated NaCl. These changes in cattle hyperimmunised intramuscularly are of the same order of magnitude as those found by Hartley for cattle successfully hyperimmunised by the subcutaneous route. Further, it is very clearly demonstrated that these changes in the intramuscularly hyperimmunised oxen are greater in magnitude and are a more constant feature than are the changes of these protein fractions in cattle hyperimmunised intrarumenally with either virulent blood or the extract of pulped virus-containing organs.

With the intrarumenal injection of virulent fluid the changes of the euglobulin proteins may be as great as those in the sera of cattle hyperimmunised intramuscularly or subcutaneously. On the other hand, other animals show an increase of the proportions of euglobulin no greater than that produced by the intrarumenal injection of normal blood, or of diluted colostrum. This is

particularly true when virulent blood is given intrarumenally, though a very small increase of serum euglobulin can, in some cases, follow the intrarumenal injection of the virulent extract of pulped organs.

If a large increase of the globulins, and in particular of the relative proportions of euglobulin, can be taken as indicative of successful hyperimmunisation, then the above results lend further support to the view that the intrarumenal hyperimmunisation of cattle does not produce such potent antiviral serum as that obtained by the more established methods. Further, the inferiority of intrarumenally-prepared serum would seem to be due to the almost complete failure to hyperimmunise some cattle whilst others are hyperimmunised perfectly satisfactorily.

Hornby (1926) showed that the thick stratified epithelium which lines the mouth, oesophagus, etc., affords ample protection against rinderpest virus and penetration of the virus cannot take place unless the lining is broken. He states that the occasional infections set up by drenching a susceptible beast with virulent blood result from " penetration " of the wall of the pharynx or after passing the first three stomachs. The injection of a massive dose of virulent material into the rumen would therefore, it seems, have to pass into the abomasum before absorption can take place. Rinderpest virus is easily destroyed at body heat, and so it is easy to visualise the rapid destruction of much of the virulent material by the rumenal juices and fauna or their alteration so that the virus is no longer antigenic. If the virulent material does not pass through to the abomasum quickly the probability is that little absorption of virus can take place, and an inferior antibody response will result.

In his preliminary work Hornby (1922) states that more virus needs to be given *via* the alimentary tract (intrarumenally or by drenching) than by the intravenous or intraperitoneal routes if antisera of equal titre are to be obtained.

A review of the work done on the chemical nature of antibodies such as that given by Marrack (1934) leaves little doubt as to the protein nature of antibodies. Their precipitation with the globulin fraction of antisera indicates that they possess the properties of globulins. The work of many workers with various antigens indicates that a considerable increase of globulin takes place during immunisation and hyperimmunisation when the antibody content of the serum is greatly increased. In this connection the results of Hiss and Atkinson (1901), Gibson and Banzhaf (1910), Hartley (1914), Reymann (1924), Kirkbride and Murdick (1927), Ledingham, Morgan and Petrie (1931) and Laidlaw and Dunkin (1931) may be cited. However, though most of the above workers found that increase of globulin took place with increase of antibody, yet the two things did not run parallel, and a few anomalous cases were encountered.

In view of the large supporting evidence from the literature we may assume that, since the intramuscular method gives a large relative increase of euglobulin, the antiviral content is also fairly high. Further, the indications are that, though the response to the intrarumenal injection of virulent fluid may be as high as that to the intramuscular injection, yet a large number of cattle given virulent blood intrarumenally appear to be only poorly hyperimmunised and perhaps not hyperimmunised at all.

Conclusions.

(I) An increase of total protein, total globulin, euglobulin and globulin insoluble in saturated NaCl takes place as a result of intramuscular hyperimmunisation of cattle against rinderpest virus. The relative increase of the more insoluble globulins is of the same order as that found by Hartley for cattle successfully hyperimmunised by the subcutaneous route.

(II) The changes in the serum proteins of cattle given a massive dose of virulent blood intrarumenally can be as high as those for cattle hyperimmunised intramuscularly or subcutaneously. A large number of animals, however, show changes in the serum proteins no greater than those found in cattle given normal blood or diluted colostrum intrarumenally.

(III) When the aqueous extract of pulped virus-containing organs is given intrarumenally the serum protein changes can be as high as those obtained with intramuscularly hyperimmunised cattle. Individual cases are found, however, in which only a relatively small increase of the euglobulins takes place.

(IV) The results obtained indicate that in certain cases an increase of the pseudoglobulin proportions may take place as well as the normal increase of euglobulin as a result of hyperimmunisation.

(V) In so far as globulin increase may be indicative of antibody formation, the results support the view that antisera made by the intrarumenal method are inferior to those prepared by the more established techniques.

Acknowledgments.

It is with great pleasure that I acknowledge the help received from Mr. S. A. Evans, M.R.C.V.S., during these experiments. All the animals were under his professional charge and he carefully supervised the hyperimmunising and bleeding of all animals. My thanks are also due to Mr. H. E. Hornby, for his advice and criticisms on many occasions.

References.

Béclère, Chambon and Menard. (1899). *Ann. Inst. Past.*, **13,** 81.
Bennet and Evans. (1934). *J. Comp. Path. Ther.*, **47,** 87.
Findlay. (1931). *Brit. J. Exp. Path.*, **12,** 9.
Gibson and Banzhaf. (1910). *J. Exp. Med.*, **12,** 411.
Haddow, Sen and Roy. (1933). *Ind. J. Vety. Sci.*, **3,** 149.
Hall. (1929). *Ann. Rept. Vety. Pathologist., N. Nigeria.*
——. (1933). Investigations on Rinderpest Immunisation.
Hartley. (1914). *Mem. Dept. Agric., India*, **1,** 221.
Hiss and Atkinson. (1901). *J. Exp. Med.*, **5,** 47.
Hornby. (1922). *Ann. Rept. Dept. Vety. Sci., Tanganyika.*
——. (1926). *Vet. J.*, **82,** 348.
——. (1927.1). *Ibid.*, **83,** 155.
——. (1927.2). *Ibid.*, **83,** 262.
Kirkbride and Murdick. (1927). *J. Immunol.*, **14,** 235.
Laidlaw and Dunkin. (1931). *J. Comp. Path. Ther.*, **44,** 1.
Ledingham. (1907). *J. Hyg.*, **7,** 65.
Ledingham, Morgan and Petrie. (1931). *Brit. J. Exp. Path.*, **12,** 357
Maitland and Burbury. (1927). *J. Comp. Path. Ther.*, **40,** 93.
Marrack. (1934). The Chemistry of Antigens and Antibodies.
Morgan and Fairbrother. (1930). *Brit. J. Exp. Path.*, **11,** 512.
Reymann. (1924). *Z. Immunforsch.*, **30,** 507.
Weyer, Park and Banzhaf. (1929). *Amer. J. Path.*, **5,** 517.

THE SERUM PROTEIN CHANGES DURING THE IMMUNISATION OF GOATS AGAINST RINDERPEST VIRUS.

By M. H. French, M.A., DIP. AGRIC. (CANTAB.).

Antibody formation is almost always associated with an increase of the serum globulins. Since serum globulin was assumed to consist of two fractions investigators have sought to locate the antibody in one or other of these fractions. It soon became evident that antibodies were not confined to one fraction only, and efforts at concentration became directed to the removal of antibodies with the globulins precipitated between two different salt concentration levels.

Most of the investigations on the distribution of antibodies among the serum protein fractions have been carried out with antitoxins, and in particular diphtheria antitoxin. Pick (1901) confirmed the work of Dieudonne (1897), Belfanti and Carbone (1898), and Seng (1899) that anti-diphtheria toxin was associated with the pseudoglobulin fraction of horse serum. Ledingham (1907) found that the euglobulin fraction of horse serum showed the greatest increase during immunisation. Gibson and Banzhaf (1910) found that both fractions could be affected in quantity during immunisation against diphtheria toxin. Barr (1932) has shown that diphtheria antitoxin is present in both pseudoglobulin and euglobulin (water insoluble) fractions, and that this is true for the sera of several animal species.

Similarly, with antiviral bodies Ledingham, Morgan and Petrie (1931) have shown that the anti-vaccinial body of horse serum is associated with both globulin fractions. The work of Haddow, Sen and Roy (1933) indicates that this is true for ox anti-rinderpest serum.

Not only may an antibody appear in more than one protein fraction but, as the results of Barr, Glenny and Pope (1931), and Barr and Glenny (1931) show, the distribution of antibody may be altered by the addition of normal serum euglobulin or albumin, or by blending one serum with another of a different fractional protein composition. The separation of antibody from a serum therefore appears to depend on the relative amounts and distribution of the different serum protein fractions in the serum.

If this is true we should expect to find that the distribution of a given antibody was not the same in the serum from different animal species. There are several references in the literature to show that this is the case. Pick (1901) showed that diphtheria antitoxin was associated with the pseudoglobulin fraction in horse serum but with the euglobulin fraction of goat serum. Banzhaf and Famulener (1914) found the diphtheria antitoxin to be present

in both fractions of goat serum globulin, but that the euglobulin fraction contained more than that of the pseudoglobulin. The anti-pneumococcal bodies of horse serum can be separated with the small " Felton fraction " of the serum globulins [Felton (1925, 1928, 1931)], but this is not so for this antibody in rabbit serum [Heidelberger and Kendall (1933)].

Barr (1932) has made the most complete study of the variation of a given antibody in sera from different animal species. She found great variations in the salting out limits for diphtheria antitoxin from the sera of different species, when the precipitation conditions were kept constant. She found the limits of antibody precipitation for sheep and guinea-pig sera were close together, and that the difference between the higher and lower salt concentration levels became wider with cow and rabbit sera, whilst with goat serum the antibody was distributed over a very wide range of proteins as judged by the salting out concentrations. An attempt has been made to find out what difference of distribution of anti-rinderpest antibody occurs in ox and goat sera.

Protein Changes in Goats during Immunisation Against Rinderpest Virus.—The serum protein changes during the immunisation of four goats has been studied and the results are given in the Tables I to IV. In each case the goats were inoculated with 10 c.c. citrated virulent blood without any protective dose of antiserum. In each case the animals underwent a mild rinderpest reaction, with a high thermal reaction but no clinical symptoms. The methods of analysis were the same as those used in studying the changes in ox serum [French (1936)].

TABLE I.

Serum Protein changes during Immunisation. (Goat K.)

	22.5.35.	28.5.35.	3.6.35.	8.6.35.	12.6.35.
Total N per cent.	1·1102	1·0845	1·0500	1·1410	1·1452
Non-Protein N per cent....	0·0323	0·0345	0·0294	0·0235	0·0353
Total Protein N per cent....	1·0779	1·0500	1·0206	1·1175	1·1099
Euglobulin N per cent. ...	0·0749	0·1105	0·1481	0·2153	0·2804
Pseudoglobulin N per cent.	0·4831	0·4855	0·5568	0·5319	0·4550
Total Globulin N per cent.	0·5580	0·5960	0·7049	0·7472	0·7354
Albumin N per cent. ...	0·5199	0·4540	0·3157	0·3703	0·3745
N per cent. of Protein insoluble in saturated NaCl	0·1974	0·1887	0·1624	0·2366	0·3332
Albumin as per cent. of Protein	48·23	43·24	30·93	33·14	33·74
Pseudoglobulin as per cent. of Protein	44·81	46·24	54·55	47·60	40·99
Euglobulin as per cent. of Protein	6·95	10·52	14·52	19·26	25·26
Saturated NaCl insoluble Protein as per cent. of total Protein	18·31	16·07	15·92	21·17	30·02

TABLE II.

SERUM PROTEIN CHANGES DURING IMMUNISATION. (GOAT P.)

	22.5.35.	28.5.35.	3.6.35.	8.6.35.	12.6.35.
Total N per cent.	1·0570	1·0724	0·9996	1·1074	1·0038
Non-Protein N per cent....	0·0323	0·0323	0·0441	0·0235	0·0353
Total Protein N per cent....	1·0247	1·0401	0·9555	1·0839	0·9685
Euglobulin N per cent. ...	0·1110	0·0818	0·1714	0·1817	0·1390
Pseudoglobulin N per cent.	0·3938	0·3532	0·4831	0·4832	0·4547
Total Globulin N per cent.	0·5048	0·4350	0·6545	0·6649	0·5937
Albumin N per cent. ...	0·5199	0·6051	0·3010	0·4190	0·3748
N per cent. of Protein insoluble in saturated NaCl	0·1546	0·1456	0·1932	0·2050	0·1614
Albumin as per cent. of Protein	50·74	58·18	31·50	38·65	38·70
Pseudoglobulin as per cent. of Protein	38·43	33·96	50·56	44·57	46·95
Euglobulin as per cent. of Protein	10·82	7·86	17·94	16·77	14·35
Saturated NaCl insoluble Protein as per cent. of total Protein	15·08	14·00	20·22	18·91	16·66

TABLE III.

SERUM PROTEIN CHANGES DURING IMMUNISATION. (GOAT R.)

	17.4.35.	22.4.35.	28.4.35.	2.5.35.	8.5.35.
Total N per cent.	1·0948	1·2109	1·0041	1·1718	1·1480
Non-Protein N per cent....	0·0323	0·0358	0·0323	0·0323	0·0323
Total Protein N per cent....	1·0625	1·1751	0·9718	1·1395	1·1157
Euglobulin N per cent. ...	0·1123	0·1276	0·0822	0·1121	0·1680
Pseudoglobulin N per cent.	0·3816	0·3554	0·3860	0·5075	0·5075
Total Globulin N per cent.	0·4939	0·4830	0·4682	0·6196	0·6755
Albumin N per cent. ...	0·5686	0·6921	0·5036	0·5199	0·4402
Albumin as per cent. of Protein	53·52	58·90	51·81	45·62	39·45
Pseudoglobulin as per cent. of Protein	35·92	30·24	39·72	44·54	45·48
Euglobulin as per cent. of Protein	10·52	10·86	8·46	9·84	15·06

TABLE IV.

SERUM PROTEIN CHANGES DURING IMMUNISATION. (GOAT S.)

	17.4.35.	22.4.35.	28.4.35.	2.5.35.	8.5.35.
Total N per cent.	1·1396	1·1369	1·0926	1·0971	1·1220
Non-Protein N per cent....	0·0460	0·0358	0·0342	0·0339	0·0385
Total Protein N per cent....	1·0936	1·1011	1·0584	1·0632	1·0835
Euglobulin N per cent. ...	0·1124	0·1093	0·1415	0·1865	0·1500
Pseudoglobulin N per cent.	0·4060	0·4279	0·4841	0·5051	0·4899
Total Globulin N per cent.	0·5184	0·5372	0·6256	0·6916	0·6399
Albumin N per cent. ...	0·5752	0·5639	0·4328	0·3716	0·4436
Albumin as per cent. of Protein	52·60	51·21	40·89	34·95	40·94
Pseudoglobulin as per cent. of Protein	37·13	38·86	45·74	47·51	45·22
Albumin as per cent. of Protein	10·27	9·93	13·37	17·54	13·84

These figures indicate that the total proteins of goat serum show a temporary fall during immunisation but later return to a high level. This is parallel to what happens in cattle during immunisation with antiserum. The total globulins do not show a constant temporary fall similar to that of the total proteins, though this happens in two cases. In every case the total globulins rise to a high level by the end of the second week after infection. The albumin content falls to about the end of the second week and then rises slowly.

The changes in the euglobulin content are not quite uniform, in that a temporary drop may or may not be observed but in all cases the content had increased at the end of a fortnight. The pseudoglobulin changes are similar but in this case a very much bigger increase is observed than was seen for the euglobulin fraction. The fraction insoluble in saturated NaCl is greater in amount than the euglobulin fraction but the changes show a temporary fall and then a big rise later.

The relative proportions of the various fractions show that the proportional pseudoglobulin increase is somewhat greater than that of the euglobulin fraction. This is the reverse of that found for the changes in cattle [Hartley (1914), Sen and Roy (1933) and French (1936)], where the biggest proportional increase is in the euglobulin protein and the pseudoglobulin increase is a less constant feature.

Since the antiviral body of ox serum is associated with the globulin fractions [Hartley (1914), and Haddow, Sen and Roy (1933)], we may assume that the antibody of goat serum is likewise associated with the serum globulins. This means that in the goat the distribution of the antivirus is spread over a wider serum protein range (as determined by Na_2SO_4 precipitation) than it is in the ox. This result is the same as that found by Barr (1932) for anti-diphtherial toxin.

Summary.

(1) The serum protein changes during immunisation of a goat against rinderpest are presented.

(2) In the goat the serum globulins show a big increase similar to that occurring in the immunisation of the ox, but in the goat the pseudoglobulin increase is much more than that found in the ox, whilst the reverse is true for the euglobulin fraction.

(3) The indications are that the antiviral body is spread over a wider serum protein range in the goat than in the ox.

Acknowledgments.

The animals used in this investigation were supplied by my colleague, Mr. S. A. Evans, M.R.C.V.S. I am also indebted to him for the clinical observations and for general help and advice.

References.

Banzhaf and Famulener. (1914). *Coll. Stud. Dept. Health, N.Y. City*, **8**, 208.
Barr. (1932). *J. Path. Bact.*, **35**, 913.
Barr and Glenny. (1931). *Brit. J. Exp. Path.*, **12**, 337.
Barr, Glenny and Pope. (1931). *Ibid.*, **12**, 217.
Belfanti and Carbone. (1898). *Zeit. f. Bakt.*, **23**, 906.
Dieudonne. (1897). *Arb. Kaiserlichen. Ges.*, **13**, 293.
Felton. (1925). *J. Infect. Dis.*, **37**, 119 and 307.
——. (1928). *Ibid.*, **42**, 248.
——. (1931). *J. Immunol.*, **21**, 357.
French. (1936). In Press.
Gibson and Banzhaf. (1910). *J. Exp. Med.*, **12**, 411.
Haddow, Sen and Roy. (1933). *Ind. J. Vety. Sci.*, **3**, 149.
Hartley. (1914). *Mem. Dept. Agric. India*, **1**, 178.
Heidelberg and Kendall. (1933). *J. Exp. Med.*, **57**, 373.
Ledingham. (1907). *J. Hyg.*, **7**, 65.
Ledingham, Morgan and Petrie. (1931). *Brit. J. Exp. Path.*, **12**, 357.
Pick. (1901). *Beitr. Chem. Physiol. u. Path.*, **1**, 351.
Seng. (1899). *Zeit. f. Hyg.*, **31**, 573.
Sen and Roy. (1933). *Ind. J. Vety. Sci.*, **3**, 39.

A *BR. ABORTUS* SUSPENSION OF UNIFORM AGGLUTINABILITY STANDARDISED BY MEANS OF A DRY STABLE STANDARD ANTI-ABORTUS SERUM.

By A. W. Stableforth,
Department of Preventive Medicine, Royal Veterinary College, London.

The object of this report is to describe the preparation of a suspension of *Br. abortus* which is standardised to uniform agglutinability by means of a dried anti-abortus serum.

A suspension of uniform agglutinability has the advantage that, provided the method of testing is the same, results obtained with it at any time or place are directly comparable. The course of the titre in a given animal over a long period or the titre produced by two vaccines used at widely separated intervals can thus be closely followed, whilst discussion of the significance of agglutination at a certain titre is simplified.

Some method of standardisation is necessary, because strains of *Br. abortus*, like other bacteria, cannot be relied on to keep their properties unchanged for long periods. Moreover, suspensions made in different ways from the same strains, or, as will be shown here, even by the same method in different laboratories, may not have the same agglutinability. Finally, a strain selected for suspension preparation may become rough or inagglutinable, or may be lost and have to be replaced by another of unknown and possibly different agglutinability.

In order to standardise suspensions it is desirable to have a standard serum which will retain a constant titre indefinitely or for a considerable period. Natural undried sera are often used for this purpose and it is known that if unpreserved and sterile they retain their titre for a long period, but in time they deteriorate. They may also become contaminated, whilst, if a preservative such as phenol is added, deterioration is more rapid and inconvenient inhibition zones may develop. These difficulties are obviated by the use of a stable dried serum, such as that used here. There is now ample evidence from observations on antisera of various kinds that when serum is dried quickly to constant weight *in vacuo* its antibody content remains unaltered, apparently indefinitely.

The standardised suspension to be described is that used by all laboratories embraced by the experimental scheme of the Agricultural Research Council (Gt. Britain) for the control of contagious abortion by segregation of infected animals and in most other laboratories doing similar work in this country.

General Methods Used.

Since the actual methods used in different laboratories for agglutination tests vary considerably (Stableforth, 1936) a brief outline of the general methods adopted in this laboratory is given.

Serum dilutions are made by the usual serial method, seven dilutions being put up with each serum, *viz.*, final dilutions 1 : 10 to 1 : 640. Tubes measuring approximately 3 inches by ½ inch (7·5 cm. by 1·2 cm.) are used. Into the first tube is placed 1·6 c.c. of 0·5 per cent. carbol-saline and into each of the remainder 1·0 c.c. To the saline in the first tube is then added 0·4 c.c. of whole serum. The contents of the tube are well mixed by means of a pipette and 1·0 c.c. of the mixture carried to the second tube. The process is repeated until the last tube is reached where, after mixing, 1 c.c. is discarded. To these 1 c.c. volumes of serum dilutions, *viz.*, 1 : 5 to 1 : 320, 1 c.c. of suspension is then added, giving a final series of dilutions ranging from 1 : 10 to 1 : 640. Tests are incubated at 37° C. for 20 hours. Routine tests are read by ordinary daylight against a wall or other darkish background and recorded as :—

+ + + (complete or nearly complete clearing and sedimentation),
+ + (about 50 per cent. clearing and marked sedimentation), and
+ (about 25 per cent. clearing and distinct sedimentation), the sign
± being used where reasonable doubt exists whether any significant agglutination is present.

The Dry Standard Anti-Abortus Serum.

The serum used for the preparation of the dry standard was obtained in October 1933 from a cow naturally infected with *Br. abortus*, whose serum agglutinated a suspension of a strain of average agglutinability at the chosen opacity to a titre between 1 : 320 and 1 : 640. About 500 c.c. of serum was passed through a Seitz filter and found to be sterile.

By means of a precision distribution apparatus exactly 2·5 c.c. quantities of the sterile, preservative-free serum were delivered under aseptic conditions into a series of sterile 5 c.c. ampoules, and each ampoule then closed with a sterile plug of cotton-wool. A few ampoules were sealed and stored in order that comparison might be made with the dried product immediately after drying and after various periods of storage. The contents of the remaining ampoules were reduced to absolute dryness by exposure to the dehydrating action of phosphorus pentoxide at low pressures (1 mm. or less), the desiccation process being continued until the weights of selected ampoules attained a constant value. The ampoules were then filled with pure, dry nitrogen gas and finally sealed. The ampoules, 184 in all, were transferred to cold storage (−2° C. to 4° C.) and are maintained constantly at this temperature.

Reconstruction of Serum.

An ampoule is opened in the ordinary way, care being taken that no particles of dried serum are lost. Ordinarily, ampoules are kept in the upright position so that no particles reach the neck of the ampoule. Two-and-a-half c.c. of sterile distilled water are then added to the dried serum and the ampoule and contents placed at 37° C. for 30 to 60 minutes. The ampoule is then rotated until a homogeneous, though slightly turbid, solution is obtained.

The whole of the contents is then made up directly to 400 c.c. with 0·5 per cent. carbol-saline in a measuring cylinder, the ampoule and, if necessary, the end which has been removed being washed out. The dilution of 1 : 160 thus obtained was chosen because the serum has a titre of between 1 : 320 and 1 : 640 with the average suspension. Further dilutions are prepared as follows :—

Dilution required :	1 : 160	1 : 200	1 : 240	1 : 280	1 : 320
c.c. of 1 : 160 dilution	100	80	80	80	50
c.c. of carbol-saline	0	20	40	60	50

giving, when mixed with 1 c.c. of suspension in the usual way, final dilutions of 1 : 320, 1 : 480, 1 : 560 and 1 : 640.

Comparison of Parent and Reconstructed Dried Serum.

The first comparative titration of the parent and of the reconstructed dried serum (ampoules A and B) was carried out a few days after the drying was completed (7.12.33) with the results given in Table I. Three suspensions of slightly different agglutinability were chosen for the test, two of which were slightly below and one slightly above the sensitivity eventually decided upon for standard suspension.

TABLE I.

Comparison of Natural and Dried Anti-Abortus Serum, in December, 1933.

Suspension.	*Serum.*	*Dilution of Serum.*				
		1 : 320	1 : 400	1 : 480	1 : 560	1 : 640
III	Parent serum	+++	+	—	—	—
„	Dried serum ampoule A	++	±	—	—	—
„	„ „ „ B	++	±	—	—	—
V	Parent serum	+++	++	—	—	—
„	Dried serum ampoule A	+++	+	—	—	—
„	„ „ „ B	+++	+	—	—	—
G 2	Parent serum	+++	+++	+++	+	—
„	Dried serum ampoule A	+++	+++	++	—	—
„	„ „ „ B	+++	+++	++	±	—

It is seen from Table I that the reconstructed serum from both ampoules of dried serum gave a very slightly lower titre than the parent serum with each of the three suspensions, but that the loss was of no importance, and that the two ampoules of dried serum gave exactly the same value with two suspensions whilst the difference in the case of the third was within the limits of experimental error. Sets of dilutions made up from two different ampoules of dried serum have been compared on several occasions since the experiment detailed above and in no case has any significant difference in titres been found.

Table II shows a comparison made on January 14th, 1936, of : (1) the parent unpreserved serum, (2) the dried serum reconstructed

TABLE II.

Comparison of Parent Serum (Annabel) diluted 14.1.36; Dry Standard reconstructed and diluted, 14.10.35 (Serum J); and Dry Standard reconstructed and diluted, 14.1.36 (Serum K).

Serum.	Suspension.	Serum Dilutions. 1 : 320	1 : 400	1 : 480	1 : 560	1 : 640
Parent serum, series (*a*)	... 34*a*	+++	+±	+	—	—
„ „ „ (*b*)	... 34*a*	+++	++	+	±	—
„ „ „ (*c*)	... 34*a*	+++	++	+	±	—
Dried „ J „ (*a*)	... 34*a*	+++	++	+	±	—
„ „ „ (*b*)	... 34*a*	+++	++	+	±	—
„ „ „ (*c*)	... 34*a*	+++	++	+	±	—
„ „ K „ (*a*)	... 34*a*	+++	++	+	±	—
„ „ „ (*b*)	... 34*a*	+++	++	+±	±	—
„ „ „ (*c*)	... 34*a*	+++	++	+	±	—
Parent serum (one series only)	... 33	+++	++	+	—	—
Dried „ J (one series only)	... 33	+++	++	+	—	—
„ „ K (one series only)	... 33	+++	++	+	—	—
Parent serum (one series only)	... 32	+++	++	+	—	—
Dried „ J (one series only)	... 32	+++	++	+	—	—
„ „ K (one series only)	... 32	+++	+++	+	—	—
Parent serum, series (*a*)	... 32*a*	+++	+±	+	—	—
„ „ „ (*b*)	... 32*a*	+++	++	+	—	—
„ „ „ (*c*)	... 32*a*	+++	++	+	—	—
Dried „ J „ (*a*)	... 32*a*	+++	++	+	—	—
„ „ „ (*b*)	... 32*a*	+++	++	+	—	—
„ „ „ (*c*)	... 32*a*	+++	++	+	—	—
„ „ K „ (*a*)	... 32*a*	+++	++	+	—	—
„ „ „ (*b*)	... 32*a*	+++	++	+	—	—
„ „ „ (*c*)	... 32*a*	+++	++	+	—	—

Dried serum J means ampoule J ; each ampoule and the dilutions from it being given a serial letter.
Series *a*, *b* and *c* are the three series of tests of those tested in triplicate.

and diluted immediately before use and (3) the dried serum reconstructed and diluted three months previously. The latter was included because these dilutions were being used for testing weekly the suspensions being issued from the laboratory; for this purpose it is, of course, impracticable to use an ampoule of dried serum on every occasion. The three sets of serum dilutions were tested with each of four suspensions, three of which were of standard sensitivity, whilst the fourth was fairly freshly prepared and still slightly above standard sensitivity. Two of these tests were made in triplicate. It is seen that no significant difference existed at this date (14.1.36).

Comparison of Tables I and II suggests that, whilst the dried serum had maintained its titre, the titre of the parent serum had dropped slightly in the interval of just over two years between the two tests. The differences are small, hardly significant, but all point in the same direction.

Preparation and Standardisation of Suspensions.

Standard suspension is prepared from a single strain of *Br. abortus* (B22) of average agglutinability isolated from a bovine fœtus in the spring of 1933. It was originally CO_2-sensitive, but when the preparation of a standard suspension was begun in September 1933, it already grew well in atmospheric air. The stock strain has been subcultured heavily on potato agar or liver agar 15 times since September 1933, *i.e.*, about once every two to three months. Other subcultures have been made when required for the preparation of suspension. The bacteria for suspension are grown on ox liver agar, *p*H 7·0, in Roux flasks, 100 c.c. of agar being heavily seeded with an actively growing culture and incubated for three days at 37° C. The growth is then washed off in a small volume of 0·85 per cent. saline. Films are examined from each flask and any growths which show organisms other than *Br. abortus* are discarded. The thick suspension is then bulked, heated for one hour at 60° C. and filtered through glass wool. At the same time a test of thermostability (Wilson, G. S., 1933) is carried out as one means of ensuring the smoothness of the strain. For this purpose about 5 c.c. of thick suspension, of an opacity approximately ten times that of tube 10 of Brown's $BaSO_4$ standard opacity series, is placed in a 3 in. × ½ in. test tube and heated in a boiling water bath for two hours. In no case has sedimentation occurred, indicating as far as this one test is able that the strain is still in the smooth phase. The thick suspension is then carbolised to 0·5 per cent. and diluted to an opacity between that of tubes 4 and 5 of Brown's series with 0·5 per cent. carbol-saline.

Standardisation of Suspensions.

The results obtained with the first few suspensions prepared from the selected strain showed that at an opacity corresponding to Brown's tube 4 (giving a serum-suspension opacity equal to Brown's tube 2), all were agglutinated with standard dried serum to a titre between 1 : 320 and 1 : 640, usually approximately 1 : 480 and that, when slight variation from this occurred, the titre could be adjusted by slight alteration of the opacity. The degree of variation in titre with varying bacterial content, *i.e.* opacity, in the case of *Br. abortus* has been dealt with elsewhere (Stableforth, 1936). Table III, taken from the paper referred to, shows the approximate relation of the titres obtained with suspensions varying in opacity from that of Brown's tube 4 to two-fifths that of Brown's tube 1 (−2/5 in Table III), the titre with suspension of the thickness of Brown's tube 4 being taken as 1·0. It was decided, therefore, that the standard suspension issued should be of uniform agglutinability, and that the standard adopted should be + or approximately 25 per cent. agglutination, with the reconstructed standard dried serum at a dilution of 1 : 480, and that the

TABLE III.

RELATION OF TITRES GIVEN BY SIX SERA WITH NINE SUSPENSIONS OF DECREASING OPACITY.

	Opacity in Terms of Brown's $BaSO_4$ Tubes.								
Suspension...	4	3½	3	2½	2	1½	1	½	⅖
Serum-suspension mixture ...	2	1¾	1½	1¼	1	¾	½	¼	⅕
Corrected relation	1·0	1·15	1·30	1·45	1·60	2·16	2·72	3·70	4·3

opacity should not vary more than half a tube on either side of the opacity of Brown's tube 4 and in no case be less than tube 3 or more than tube 5. It has been found that, up to the present, adopting the same method of preparation throughout and sowing 50 to 100 flasks at a time, there has been little difficulty in keeping to these criteria. Only occasionally has a suspension of such high or low agglutinability been obtained that mixing of different batches has become necessary.* The earlier suspensions were also tested with stock abortus sera which had titres of 1 : 10, 1 : 20 and 1 : 40 respectively, and it was found that suspensions which had been standardised to the same end-point with the close dilutions of the standard dried serum were agglutinated similarly by the lower titred sera. Instead of issuing a suspension of standard agglutinability, it might have been decided to issue suspensions as obtained and to accompany them with a sensitivity factor. Had the difference in the agglutinability of suspensions been great this would have been necessary, but, for reasons to be given later, it was desired to avoid the use of a factor.

Suspensions Prepared in the Different Laboratories.

It has already been stated that the suspension referred to in this report was prepared primarily for use in the experimental abortion eradication scheme of the Agricultural Research Council (Gt. Britain). At first, suspension was prepared by each laboratory requiring it from the same strain which was kept at this laboratory. Subcultures were sent on request, the suspension prepared by the agreed method and a sample of it sent to this laboratory for test with the standard serum in order to ensure comparable results. It was found, however, that although the same strain and similar methods were used there was some difference in the sensitivity of the suspensions obtained. This was generally not more than one tube in the usual series of doubling dilutions, but occasionally wider differences occurred, the most sensitive suspension being

*Since this report was written the original strain has become less agglutinable and ceased to give a sharp end point. It has, therefore, been replaced by another strain with which there is no difficulty in maintaining the standard agglutinability at the selected opacity.

nearly four times as agglutinable as the least sensitive. Moreover, as shown in Table IV, the differences were great enough to make an important difference in the case of many sera the titres of which were on the border-line.

An attempt was made to find the explanation for these differences by comparing 36 suspensions prepared from: (*a*) growths on agar made from different livers, both fresh and imported, (*b*) growths on different flasks from the same liver and (*c*) growths on agar made from samples of bovine muscle obtained from six different butchers. All suspensions were prepared at the same time and by the same methods, adjusted to the same opacity and tested with the same dilutions of a number of different sera made up in bulk. Many were tested in triplicate, no significant variation being found. These suspensions were also compared with four suspensions received from other laboratories at that time. It would serve no useful purpose to give the results in detail, but it may be said that, whilst small, probably not significant differences in sensitivity were found among different flasks from the same liver and somewhat larger differences when different livers were used, these differences were smaller than those among suspensions prepared in different laboratories.

TABLE IV.

COMPARISON OF TITRES GIVEN BY VARIOUS SUSPENSIONS, PREPARED IN THE SAME AND DIFFERENT LABORATORIES, TESTED WITH THIRTEEN SERA.

Suspension	*Approximate Titres of Sera.*			
	1 : 640 (*One Serum.*)	1 : 160 (*One Serum.*)	1 : 40 (*Five Sera.*)	1 : 20 (*Six Sera.*)
I	+ 1 : 640	/	/	/
II	+ 1 : 640	/	/	/
III	+++ 1 : 320	++ 1 : 160	/	/
IV	+ 1 : 640	+++ 1 : 160	/	/
V	± 1 : 640	++ 1 : 160	Five 1 : 40	Six 1 : 20
V*a*	++ 1 : 640	+++ 1 : 160	„ „	„ „
V*b*	+ 1 : 640	+++ 1 : 160	„ „	„ „
V*c*	+ 1 : 640	+++ 1 : 160	„ „	„ „
V*d*	+++ 1 : 320	+ 1 : 160	Four 1 : 40, one 1 : 20	Four 1 : 20, two 1 : 10
V*e*	+++ 1 : 320	+ 1 : 160	Three 1 : 40, two 1 : 20	Three 1 : 20, three 1 : 10
V*f*	+++ 1 : 320	++ 1 : 160	Four 1 : 40, one 1 : 20	Four 1 : 20, two 1 : 10
G	+ 1 : 640	+++ 1 : 160	Five 1 : 40	Five 1 : 20, one 1 : 40
L	+ 1 : 1280	+ 1 : 3200	Two 1 : 40, three 1 : 80	Three 1 : 20, three 1 : 40
M_1	+++ 1 : 320	++ 1 : 160	/	/
M_2	++ 1 : 320	+ 1 : 160	Three 1 : 40, two 1 : 20	Two 1 : 20, four 1 : 10

N.B.—Suspensions V*a*, *b*, *c*, *d*, *e* and *f* were all from separate livers; suspension V is a mixture of equal volumes of each. Suspensions G, L, M_1 and M_2 were from other laboratories; these and suspension V were all prepared at about the same date from subcultures of the same parent two generations before.

In Table IV are summarised the results of tests of 15 of the 36 suspensions, which were made on the same day. Thirteen sera were used. Eleven of these, which reacted at approximately 1 : 40 or 1 : 20, were drawn from the samples received for routine

diagnosis on a given day and include all which thus reacted to those titres. Although few in number, they indicate the considerable divergence of titre which may be given by different suspensions though they are of the same density and prepared by essentially similar methods from the same strain, and the fact that this is greater (Suspensions V, G, L, M_1 and M_2) with suspensions made in different laboratories than with those made in the same laboratory. Whilst the results suggest that an unusually high proportion of these sera were on the border-line between dilutions, it may be noted that, taking *e.g.*, the 1 : 20 sera, one suspension (L) gave a titre of 1 : 40 with three out of six sera, whilst another (M_2) gave a titre of 1 : 10 with four of the same six sera. The category into which the serum from a given animal fell and, therefore, the fate of that animal in an eradication scheme might thus depend entirely on the suspension used. It is not suggested that the fate of a high proportion of animals would in general depend on the suspension used, though at certain tests a considerable number of animals do fall on or near the border-line.

The six suspensions made from agar prepared with muscle from six different butchers all gave practically the same titre. The use of meat-broth agar had, however, the disadvantage that the yield was only about one-third of that given by liver agar. The yield from liver agar has averaged a little over 200 c.c. of suspension from each flask containing 100 c.c. of agar.

In consequence of the variations found in the sensitiveness of suspensions prepared in different laboratories, it was decided that it would be preferable that for the purpose of the eradication scheme concerned all suspensions should be prepared in one laboratory. This does not, it is true, overcome all the differences which may occur, but in practice, when large quantities of suspension are being prepared, these cancel each other to a large extent, and in any case, if a particular brew is of notably high or notably low sensitivity, its titre can be corrected by mixing it into another suspension. As already stated, however, wide variations in sensitivity have been rare; and the adding of another suspension of different sensitivity has only been necessary with three out of 37 suspensions so far prepared. In all other cases the standard sensitivity, a titre of just 1 : 480 with the standard dried serum, has been obtained by slight variation of density. It is believed, therefore, that, apart from pipetting errors, results obtained at any time with the standard suspensions are comparable.

Keeping Properties of the Standard Suspension.

The earlier suspensions were tested daily for the first week, twice during the second week and then at weekly intervals until no longer on issue. From early 1934, 100 c.c. amounts have also been stored in the cold and at room temperature (15 to 20° C.) and tested at intervals of three to six months. Suspensions keep

well, though some decrease in agglutinability occurs. This is greatest during the first week, but becomes progressively less until, after two to four weeks, it is usually for all practical purposes negligible, the titre then remaining almost constant for at least three months, often longer. After twelve months a further slight decrease of titre is usually noticeable, but most suspensions still agglutinate to at least one-half of their original titre after storage for one to two years. No significant difference has been found between storage in the cold and at room temperature. Some variation has been found between the initial titre and stability of different suspensions, but in most cases the titre (using suspension of the opacity of Brown's tube 4) with standard dried serum has been 1 : 560 to just 1 : 640 the day after washing off. Such a suspension will usually just agglutinate at 1 : 560 three or four days later and become stabilised at + at 1 : 480 within two to three weeks.

The present routine procedure is to wash off and store for one to two weeks before diluting. The opacity is then adjusted to between tubes 4 and 5 of Brown's scale and the suspension tested. According to the results obtained, further tests are made until it is reasonably certain that the titre is stable or, if as is usual, the titre is slightly low, the opacity is decreased until a stable titre of just 1 : 480 is obtained.

Diagnostic Criteria.

In Table V are given the diagnostic criteria employed with the standard suspension described.

TABLE V.

Diagnostic Criteria used with the Standard Suspension described.

Test Result.	*Diagnosis.*	
	No Details of Pregnancy.	*Stage of Pregnancy Known.*
+ at 1 : 40	Positive	Positive
+++ „ 1 : 20	„	Positive—if more than three months pregnant, otherwise doubtful
++ „ 1 : 20	„	„ „ „ „
+ „ 1 : 20	Doubtful	Doubtful—if more than one month pregnant, otherwise negative
+++ „ 1 : 10	„	„ „ „ „
++ „ 1 : 10	„	„ „ „ „
+ „ 1 : 10	Negative	Negative
Neg. „ 1 : 10	„	„

Discussion.

The advantages of using suspensions of standardised agglutinability for carrying out agglutination tests for diagnostic or comparative purposes are generally acknowledged, because it is well known that suspensions of different strains of the same

bacterium may vary widely in agglutinability and that suspensions made in the same way from the same strain may also vary. In some species, *e.g.*, streptococci, the latter variation may be great. With *Br. abortus* it is less, but as shown here may still be important. Thus, in a group of five suspensions (Table IV), all prepared at approximately the same date and according to the same method from the same strain, but in different laboratories, one was nearly four times as agglutinable as two others, the remaining two being intermediate and of about average agglutinability. These differences were such that, as shown by the comparative tests of routine sera, they would in many cases decide the class into which a given animal fell, and thus its fate in an eradication programme. It was for this reason that it was decided that all suspensions for the experimental eradication scheme concerned should be prepared at one centre.

The dried anti-abortus serum which was prepared in order to have a serum standard of uniform antibody content over a long period has, so far as can be judged after two and a half years, undergone no deterioration. This was, of course, to be expected from the now well-established stability of other antisera dried and kept in the same way at low temperatures and protected from the action of light, moisture and oxygen. Many of these have been in use for a much longer period. One such dried serum has been tested 48 times during the past ten years and dry preparations of other antisera have been tested from 10 to 20 times; in no case has a decline in potency been demonstrated. The negligible loss of specific activity as a result of desiccation and the absence of any significant difference between the activity of different ampoules of dried serum would also be expected from observations on other antisera, and from the fact that the total solid content of individual ampoules of such dried sera is for all practical purposes constant, the variation being less than 1 per cent. (Hartley, 1936.)

Given a stable antiserum, two methods of standardisation are available: firstly, the issue of a suspension of uniform agglutinability and, secondly, the issue of suspensions as they are prepared accompanied by a factor which will enable a common " reduced titre " to be obtained. The use of a reducing factor for each batch of suspension has the advantage that, if necessary, suspensions of widely diverse agglutinability can be issued. The use of factors has for long been in vogue at the Standards Laboratory, Oxford, England. Incidentally, the Oxford factors are so adjusted for each suspension and bacterium that a " reduced titre " above 1 : 10 by the method of testing recommended is indicative of infection with that particular bacterium, so obviating the necessity of remembering a diagnostic titre for each. The necessity of reducing each observed titre by a mathematical calculation is, however, a serious disadvantage where several hundred sera have to be tested daily. It was for this reason that an attempt was made to issue a

suspension of standard agglutinability, *i.e.*, one with which a given degree of agglutination would, other things being similar, always indicate the presence of the same amount of antibody and with which, therefore, results obtained at widely separated dates would be directly comparable. In practice this was found possible, because the sensitivity of the various suspensions did not vary greatly and compensation for minor differences of sensitivity could be made by altering slightly the density of the suspension. This does not interfere with the relative accuracy of the actual readings. When batches of suspension were of notably high or low sensitivity, *i.e.*, more than 1 : 640 or less than 1 : 320 with dried serum, the standard being + at 1 : 480, they can be adjusted by the addition of other suspension of high or low sensitivity. This has, however, rarely been necessary. Should the strain at present in use be lost, become rough or for any other reason unsuitable, it can be replaced by any other normal strain which will give a stable suspension of the standard agglutinability. That is, assuming, as is at present justified, that the difference in the agglutinability of different strains by specific sera is a quantitative and not a qualitative one. The slight drop in the agglutinability of the suspensions soon after washing off and their subsequent nearly complete stability is in accord with general opinion. (Committee on Bang's Disease of the American Veterinary Medical Association, 1932, and others.) They appear to keep as well at room temperature as in the cold. The reason for using a rather thick suspension, *viz.*, Brown's tube 4 (giving a serum-suspension opacity equal to Brown's tube 2, or about that produced by mixing 3 c.c. of 1 per cent. barium chloride and 97 c.c. of 1 per cent. sulphuric acid) have been dealt with elsewhere (Stableforth, 1936). Briefly, they are easy reading and (Priestley, 1933, unpublished data) the much lower personal error in the reading of a moderately thick suspension in tubes of a size convenient for the setting up of large numbers of routine agglutination tests.

The diagnostic criteria employed with the suspension described are based on the results of general experience in the control of contagious abortion in this laboratory and in other laboratories doing similar work in this country, and are believed to be the most practicable criteria for use with the suspension and method of testing employed. They may appear unduly severe to some workers; in particular, to those who use a lower serum-suspension opacity for the test or a more agglutinable strain. Both of these factors, especially the former, vary widely and when allowance is made for these, two criteria which at first sight appear of diverse severity may be found to be nearly similar. *E.g.*, in the test as recommended by the Bureau of Animal Industry, U.S.A., a very thin suspension, made from a more highly agglutinable strain than ours, is used with the result that the titres obtained with

given sera are a little over five times that obtained by our method. Thus, their classification of animals whose sera gives a positive result at 1 : 100 as reactors is approximately similar to our own classification of pregnant animals which give a positive result at 1 : 20, as reactors. *Vice versa*, similar criteria may, of course, not mean similar severity because the difference in test methods may operate in the opposite direction. Actually, our criteria are intermediate between those of other countries with which they have been compared; considerably more severe than some and a little less severe than others (Stableforth, 1936).

Summary.

Details are given of the preparation of a dry standard anti-abortus serum by drying the serum of a cow naturally infected with *Br. abortus* to constant weight over phosphorus-pentoxide at low pressures (1 mm. or less). The antibody content of the serum was slightly lowered by drying, but has since remained constant (two and a half years after drying) and from observations on other sera is expected to remain constant for a very long period.

A description is given of the preparation of a series of suspensions of *Br. abortus* of uniform agglutinability standardised exactly by titration with close dilutions of the dried anti-abortus serum.

Thirty-seven batches of suspensions have been prepared in about two and a half years by the same method from the same strain, and little difficulty has been encountered in maintaining the standard agglutinability, *i.e.*, 25 per cent. agglutination with a 1 : 480 dilution of the reconstructed dried serum. Adjustment of the agglutinability has been made by slight alteration of the opacity of the suspension. This has always been about tube 4 of Brown's series of permanent opacity standards, *i.e.*, about twice the opacity produced by mixing 3 c.c. of 1 per cent. barium chloride and 97 c.c. of 1 per cent. sulphuric acid.

Suspensions keep well at room temperature or in the cold. Some loss of agglutinability occurs during the first one or two weeks, but suspensions issued three to four weeks after washing off can be relied on to maintain their agglutinability practically unaltered for three months or longer.

I should like to express my indebtedness to the Abortion Committee of the Agricultural Research Council, under whose ægis this work was done; to Dr. P. Hartley, who kindly prepared the dried serum; and to my colleagues Mr. S. J. Edwards, Mr. E. J. Pullinger and Mr. N. J. Scorgie.

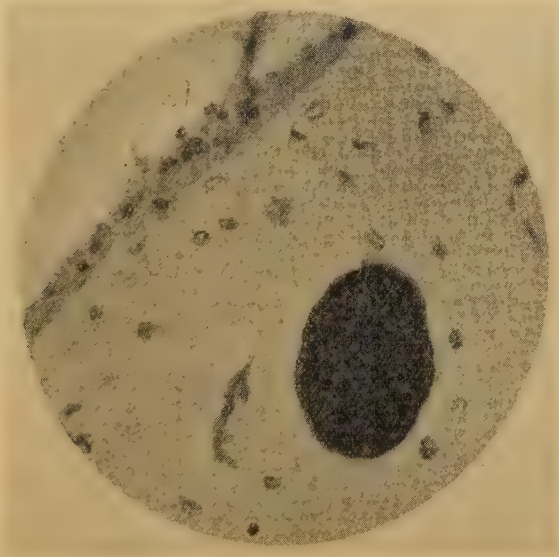
References.

Committee on Bang's Disease. (1932). *J. Amer. Vet. Med. Ass.*, **80**, 323.
Hartley, P. (1936). *Quart. Hlth. Org. L.o.N.*, **5** (in press).
Stableforth, A. W. (1936). *Bull. Off. internat. Epiz.*, **12**, 107.
Wilson, G. S. (1933). *J. Hyg.*, **33**, 516.

AN ORGANISM SHOWING THE CHARACTERS OF THE GENUS *SARCOCYSTIS* PRESENT IN THE CENTRAL NERVOUS SYSTEM OF THE SHEEP.

By A. Brownlee,
Animal Diseases Research Association,
Moredun Institute, Gilmerton, Midlothian.

During a histological examination of the central nervous system of a group of 19 sheep, which had been inoculated intracerebrally with the virus of louping-ill, cyst-like organisms were found in several sections of brain-tissues. Ten of the 19 sheep were found infected, and in these the organisms appeared to be present in very small numbers, as an examination of six serial sections of eleven parts of the central nervous system of each sheep revealed the presence of a total of only 14 cysts. (For this work the sections were cut 5μ thick and every tenth section was taken.)



Section of cerebrum of sheep showing a cyst *in situ*. ×330.

The distribution and number of cysts in each part of the brain were as follows: cerebrum, 5; cerebellum, 2; cerebral peduncles, 2; pons, 1; and medulla, including a cyst in the medullary meninges, 4. Cysts were not found in the spinal cord, although six serial sections of material taken from the cervical, thoracic and lumbar regions of the cord of each sheep were examined.

With one exception—that in which a cyst was present in the medullary meninges—all were found in brain substance, and in it they did not show a local relationship to any particular tissue element.

Description of the Cysts.

The cysts were oval, or irregularly oval, in shape. Ten cysts varied in length from 40μ to 107·3μ (average 66·9μ), and in breadth from 33·6μ to 56·4μ (average 46·1μ). The outer membrane of the cyst was usually undulating, of double contour and appeared to be radially striated when sectioned. The surface of the outer membrane of one cyst was focused and found to be marked with very minute round points, which were arranged in regularly-spaced parallel rows. It is presumably these markings which give the appearance of radial striation when the membrane is cut across. In many instances septa which divided the cyst into compartments could be faintly distinguished, and in the case of the meningeal cyst the septa were very distinct. Each compartment contained a number of closely packed oval or sausage-shaped spores.

The making of exact observations of the individual spores was rendered difficult, because the tissues in which the cysts were found had been previously fixed and the spores were closely packed together. Nineteen spores, selected from several cysts, were found to measure 9·1μ to 13·6μ long (average 10·3μ) and 2·7μ to 5·4μ broad (average 3·8μ).

All the sections were originally stained with hæmatoxylin and eosin, by means of which a few of the spores were observed to contain towards one end an oval or rounded nucleus, the contents of which were stained more faintly than the cytoplasm. The nucleus filled the short axis of the spore. A section decolorised and re-stained by iron hæmatoxylin showed similar features. Sections re-stained by Mann's method showed the spores to contain a number of very small red granules. The outline of the nucleus was very indistinct in the sections stained by this method, and it was not clear whether or not the red granules were intranuclear. The spores possessed a very delicate envelope.

One of the cysts was found to be lying in what appeared to be the remains of the host-cell, the type of which was not determined. So far as each of the other cysts was concerned, a containing cell could not be seen. No reaction was discernible in the tissue surrounding the cyst, except possibly in the case of that situated in the medullary meninges, in close proximity to which there existed two small areas of infiltration of polymorphonuclear leucocytes and, to a less extent, eosinophil leucocytes and mononuclear cells. Infiltration of inflammatory cells into the meninges was observed in other areas of the brain of this sheep, but in these the inflammatory cells were mostly of the mononuclear type and

their presence was considered to be associated with a widespread tissue-reaction to the inoculum, which had been introduced intracerebrally two days before the animal was destroyed.

Discussion.

The organisms described appear to be of a low degree of pathogenicity, as, with the one possible exception cited above, their presence did not elicit any inflammatory reaction in the surrounding tissue, and the infected sheep, before being inoculated with the virus of louping-ill, appeared to be perfectly healthy.

Two of the infected sheep had been destroyed only 24 hours after they had been inoculated with the virus of louping-ill, so that it is extremely unlikely that the infective stage of the cyst was contained in the inoculum (a suspension of sheep brain infected with the virus of louping-ill).

Morphologically, the cysts show characters strongly resembling those of the genus *Sarcocystis*. The radially striated capsule and the presence of septa are features common to both, and, although in many of the cysts the septa were not at all obvious, their indistinctness was found to be also a feature of a number of individuals of *S. tenella*. When stained by hæmatoxylin and eosin, or by Mann's method, the spores also closely resemble those of *S. tenella*. The only difference was that the spores of *S. tenella* as a rule appeared to be slightly narrower than those of the cysts under discussion. Twelve spores of *S. tenella* from several cysts in muscle-tissue, fixed by the same reagent (Susa) as was used for fixing the material under study, measured 7·3μ to 11·8μ (average 9·8μ) in length, and 2·7μ to 3·6μ (average 3·1μ) in breadth.

It is considered unlikely, however, that these cysts are those of *S. tenella* in an unusual site, as the author has examined sections of tissues from the brain of a large number of other sheep—many of which were presumably infected with sarcocysts —and no such cysts were observed in the brain of these sheep.

The group of sheep infected with these bodies had been grazing on the pasture fields of the Institute for a period varying in individual sheep from almost seven months to eleven and a half months. For a period of three years prior to the arrival of this group at the Institute, the pastures had been heavily stocked with sheep obtained from many parts of Scotland and had thus presumably become heavily infected with various parasites of sheep. The majority of sheep grazed on the pastures for only a short time, and the tissues from the brain of such as were examined microscopically did not reveal the presence of cysts. It seems probable that the sheep of the infected group during their relatively long stay had become infected with larger numbers of the cysts than usual. It appears unlikely that the sheep had contracted

infection before arriving at the Institute, as the infected individuals were derived from four different lots of sheep, obtained from three different areas of Scotland.

An apparently parallel instance of concentration of an infection is recorded by Nicolle and Manceaux (1908), who observed that in the rodent *Ctenodactylus gondi* the presence of *Toxoplasma gondii* could be demonstrated in many animals kept for a time at the Institut Pasteur at Tunis and only rarely in recently-purchased animals.

Toxoplasms have been described as occurring naturally in the brain of the following animals: dog (Mello, 1910), rabbit (Levaditi, Sanchis-Bayarri, Lepin and Schoen, 1929), guinea-pig (Nicolau, 1932), ferret (Coutelen, 1932), mouse (Nicolau and Balmus, 1933) and *Cynocephalus babuin* (Levaditi and Schoen, 1933). Though the parasites described in this paper bear some resemblance to the toxoplasms, there does not appear to be any justification for placing them in this group, since: (i) toxoplasms are not described as having a striated outer membrane, and in the preparations of toxoplasms which the author has examined the containing membrane possessed a single contour and was not striated; (ii) toxoplasm cysts are not sub-divided by septa. These features also characterise *Encephalitozoon cuniculi*.

Raillet (1895) states that Zurn found two cysts of *Balbiania gigantea* under the cerebral dura-mater of sheep. *Balbiania* is now not recognised as a separate genus and is included in the genus *Sarcocystis*. This appears to be the only previous record of the presence of a sarcocyst-like organism in the central nervous system of sheep.

Watson (1909) states that in cattle dying from suspected "Loco"-poisoning a few degenerating sarcocyst spores were found in the lung, spleen, liver and kidney. This would indicate that sarcocysts tend to degenerate when they gain access to tissues other than those of their predilection. The cysts described in this paper appeared to be healthy, so that there is no reason to believe that the brain is not the site of their predilection.

Summary.

(i) A cyst-like organism present in the central nervous system of sheep is described.

(ii) Of identified parasites, the cysts most resemble those of the genus *Sarcocystis*, but reasons are given for drawing a tentative conclusion that the cysts are not those of *Sarcocystis tenella*.

Acknowledgments.

The late Professor J. H. Ashworth very kindly examined specimens and gave me helpful criticism and advice.

I take this opportunity of thanking Mr. I. A. Galloway for giving me his

valuable opinion on the nature of these cysts and for sending me preparations of *Toxoplasma cuniculi* and *Encephalitozoon cuniculi*, Dr. Coutelen for sending me sections of tissue infected with *Toxoplasma laidlawi* and Mr. G. R. Knight for preparing the photograph.

References.

Coutelen, F. (1932). *C.R. Soc. Biol. Paris.*, cxi, 284.
Levaditi, C., Sanchis-Bayarri, V., Lepin, P., and Schoen, R. (1929). *Ann. l'Inst. Past.*, xliii, 673.
Levaditi, C., and Schoen, R. (1933). *Bull. Soc. Path. exot.*, xxvi, 402.
Mello, U. (1910). *Bull. Soc. Path. exot.*, iii, 359.
Nicolau, S. (1932). *C.R. Soc. Biol. Paris.*, cx, 763.
Nicolau, S., and Balmus, G. (1933). *C.R. Soc. Biol. Paris*, cxiii, 1002.
Nicolle, C., and Manceaux, L. (1908). *C.R. Acad. Sci.*, cxlvii, 763.
Raillet, A. (1895). Traité de Zoologie Médicale et Agricole. 2nd Edition (Paris).
Watson, E. A. (1909). *J. Comp. Path. & Ther.*, xxii, 1.

A PRELIMINARY NOTE ON THE OCCURRENCE OF INCLUSION-LIKE BODIES IN EXPERIMENTAL AND NATURAL CASES OF AFRICAN HORSE-SICKNESS AND THE PROBABLE SIGNIFICANCE OF THEIR PRESENCE IN RELATION TO THE DIAGNOSIS OF THE DISEASE.

By W. Fotheringham,
Veterinary Research Officer, Kenya Colony.

During the past 30 years workers in South and East Africa and elsewhere have devoted much time to the study of horse-sickness and the various problems associated with its occurrence and control. Some investigators have attempted to trace the source of outbreaks and to elucidate the mode of natural transmission of the virus, whilst others have sought to find a suitable susceptible small laboratory animal with which to experiment. But the line of inquiry that has claimed the attention of most investigators has had as its immediate object the development of a safe and efficient method of immunisation. More recent work on the subject has led to the discovery by Nieschulz (1932) of the susceptibility of the white mouse to the virus of horse-sickness. Alexander (1933, 1934, 1935) working independently in South Africa, confirmed and extended the findings of this author and succeeded in developing a neurotropic vaccine. Work with the same strain of virus, on which the present writer (1933, 1934, 1935) has been engaged in East Africa, has helped to confirm the main findings of these investigators, with regard both to the susceptibility of the mouse and to the attenuation for the normal host by repeated passage or neurotropic fixation in the brains of mice.

The discovery of the susceptibility of the mouse encouraged the hope that yet another aspect of the problem had been solved, that of diagnosis. A ready means of recognising horse-sickness has not, hitherto, been available, and the need for a simple method of diagnosis has long been felt in Kenya Colony. The present method of animal inoculation of suspected material is both costly and slow, and adequate supplies of suitable experimental horses are not always obtainable. It was, however, soon realised that the mouse was of little value for diagnostic purposes owing to the difficulty of establishing the virus in the earlier generations. This fact stimulated the author to continue his studies of the histo-pathology of the disease in the hope that the recognition of specific cellular changes, such as are known to occur in some other virus diseases of animals and man, might lead to a simplification of laboratory diagnosis.

The histo-pathology of horse-sickness has not been studied

extensively and the literature on the subject contains few references to the occurrence of specific cellular alterations in this disease.

In 1911 Kuhn (quoted by Rivers, 1928) described cell inclusions stainable by the Lentz method, which he found in smear preparations of the kidney from about 80 per cent. of his cases. The bodies were spherical in outline and varied in size from minute inclusions to bodies larger than the nuclei. The larger inclusions were frequently vacuolated. Similar bodies were not observed in normal horses. Carpano (1931), in a paper dealing with horse-sickness in Egypt and Eritrea, stated that he was unable to find cellular inclusions in the kidneys, as observed by Kuhn.

The object of this note is to report briefly the discovery of inclusion-like bodies in the parenchymatous cells of the liver, and to confirm the finding by Kuhn of similar bodies in the epithelial cells of the kidneys of animals infected with African horse-sickness.

The material for study was obtained from ten horses infected with horse-sickness in the course of routine vaccine production, and from two natural cases of the disease that occurred in the vicinity of Nairobi. The former had received one dose of 40 c.c. of formalised spleen vaccine 14 days prior to the injection of fully virulent virus. The diagnosis of the latter was confirmed by animal inoculation of blood preserved in oxylate-carbol-glycerine solution. In addition the tissues from two normal horses killed for reasons unconnected with this study were included as controls. Specimens of the liver and kidneys were collected immediately after death and fixed in 4 per cent. formol saline. Sections for histological examination were stained by Goodpasture's aniline-fuchsin and methylene blue. In the majority of cases attention was restricted to an examination of the liver and kidneys, as it appeared from a preliminary survey of the tissues that the more significant changes were present in these organs.

The appearance of the inclusion-like bodies in the liver cells is illustrated in Figures 1, 2 and 3. They resemble closely, both morphologically and in staining reaction, similar inclusions in yellow fever of man and Rift Valley fever of sheep; and it is probable that the process by which they are formed is alike in all three diseases.

Goodpasture's aniline-fuchsin is an excellent stain for demonstrating cytological changes in a section. When stained by this method the inclusions of horse-sickness, in common with those of the diseases mentioned, are strongly acidophilic; they stand out clearly from the rest of the tissue, and are readily detected under the microscope. They are spherical in outline and are surrounded by a distinct clear zone or halo. The size varies from quite small globules up to masses as large as a single liver cell. There may be only one (Fig. 1) or several (Fig. 2) in a cell and sometimes one

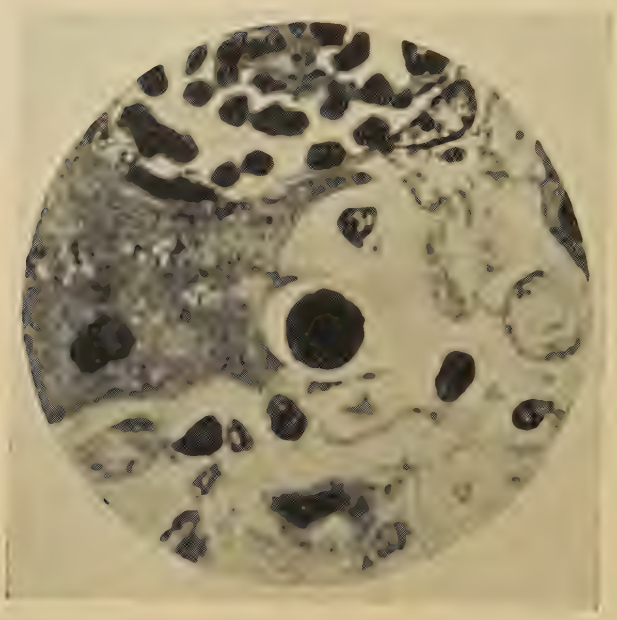


FIG. 1.

Liver. Experimental case. Hyaline body. Goodpasture's stain.

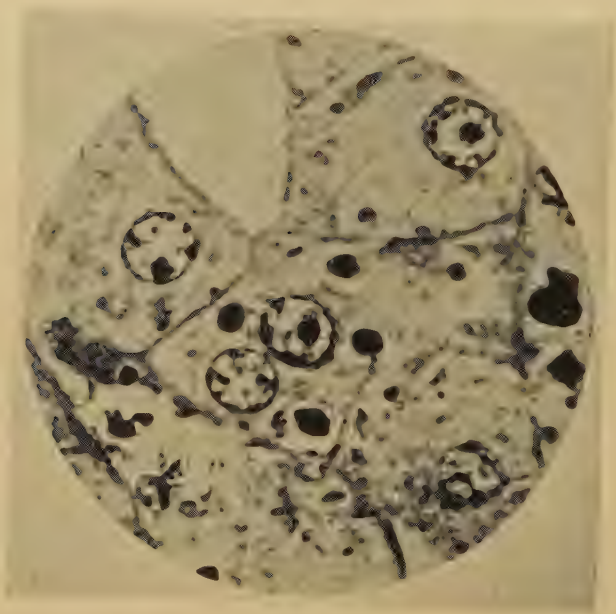


FIG. 2.

Liver. Experimental case. Smaller hyaline bodies. Goodpasture's stain.

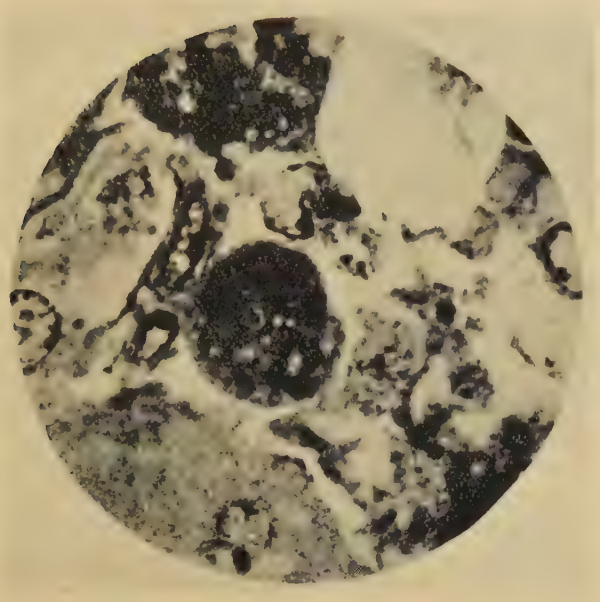


FIG. 3.

Liver. Experimental case. Large hyaline body of the "Councilman type." Goodpasture's stain.

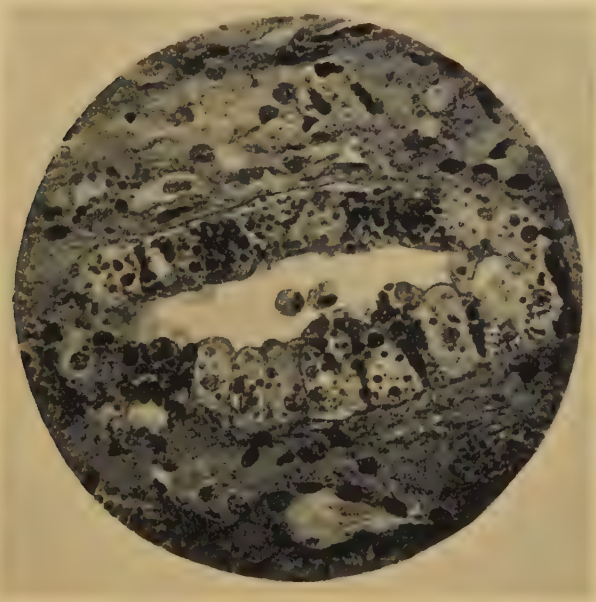


FIG. 4.

Kidney. From same experimental case as Figs. 1, 2 and 3. Granular and hyaline degeneration of cells of tubules. Goodpasture's stain.

gains the impression that the cytoplasm of a whole cell has become involved in the degenerative process, giving rise to larger globular hyaline masses (Fig. 3). The latter are frequently vacuolated, calling to mind the well-known " Councilman Lesion " of yellow fever, recently redescribed and figured by Klotz and Belt (1930).

Vacuolation, however, is not such a marked feature of the smaller and medium-sized bodies which resemble more the " hyaline bodies " of Rift Valley fever (Daubney and Hudson, 1931).

The alterations in the kidneys of animals dying from horse-sickness are no less striking than those present in the liver, and judging from the published descriptions of the renal changes in yellow fever the lesions would appear to be of the same general type in both diseases.

According to Magalhaes (1931), Councilman pointed out, as the principal lesion of the yellow fever kidney, the hyaline and granular degeneration of the cells of the tubules, in which the cytoplasm is described as being composed of immense numbers of hyaline granules, stained deeply with eosin. This is an equally good description of the renal lesions illustrated in Figure 4. Figure 4 is a photomicrograph of a kidney tubule from a case of African horse-sickness which shows the general character and intensity of the degenerative process. The cells are greatly swollen and packed with globular, hyaline, acidophilic bodies of different sizes. These undoubtedly represent the inclusions which Kuhn saw in smear preparations of the kidneys in 80 per cent. of his cases.

In addition to the lesions just described other changes play a part in the pathology of the liver and kidneys in African horse-sickness. Among these, congestion, fatty and granular degeneration and deposition of fine granules of yellowish-brown pigment within the parenchymatous cells of the liver are worthy of special mention. It is hoped to furnish at a later date a full account of all the changes that contribute to the lesions. For the purpose of this communication it is sufficient to say that the inclusion-like hyaline bodies that are figured and described were found in a number of horses experimentally infected with African horse-sickness. That they represent specific cellular changes associated with the activity of the virus of this disease seems highly probable from the fact that identical bodies were seen in two natural cases. They were not present in the tissues of two normal horses examined.

Whilst it is yet too early definitely to state that these changes are pathognomonic of horse-sickness, it seems reasonable to suppose from their presence in both the experimental and natural cases examined that they do occur with some degree of constancy in horse-sickness tissues, and that their presence when detected may well form a basis for a pathological anatomical diagnosis of the disease.

It will be necessary, however, to investigate these changes further in order to ascertain the exact distribution of the lesions (there is good reason for thinking that the hepatic lesions of horse-sickness, like those of yellow fever, may not be regularly distributed throughout all the lobes of the liver) and to determine with what degree of regularity the hyaline bodies make their appearance in animals that succumb to African horse-sickness.

References.

Alexander, R. A. (1933). Preliminary note on the Infection of White Mice and Guinea-pigs with the virus of Horse-sickness. *Jl. of S. Africa. Vet. Med. Ass.*, iv., No. 1, pp. 1-9.

Alexander, R. A., and Du Toit, P. J. (1934). The Immunisation of Horses and Mules against Horse-sickness by means of the Neurotropic Virus of Mice and Guinea-pigs. *Onderstepoort Jl. of Vet. Sc. & Ann. In.*, Vol. ii, No. 2, pp. 352-391.

Alexander, R. A., and Van der Vyver, B. (1935). Horse-sickness. Field experiments with Neurotropic Vaccine during the season 1933-34. *Jl. of S. Africa Vet. Med. Ass.*, vi, No. 1, pp. 33-38.

Alexander, R. A. (1935). Studies on the Neurotropic Virus of Horse-sickness. 1, Neurotropic Fixation; 2, Some Physical and Chemical Properties; 3, The Intracerebral Protection Test and its Application to the Study of Immunity; 4, The Pathogenesis in Horses. *Onderstepoort Jl. of Vet. Sc. & Ann. In.*, Vol iv, No. 2, pp. 291-388.

Carpano, M. (1931). African Horse Sickness as observed particularly in Egypt and in Eritrea. Bull. No. 15, Ministry of Agriculture.

Councilman, W. T. (1890). Extract from the Report on the Etiology and Prevention of Yellow Fever, by George H. Sternberg, U.S. Marine Hospital Service, pp. 151-159. Quoted by Magalhaes, A. (1931) in *Arch. of Path.*, ii, pp. 561-573.

Daubney, R., and Hudson, J. R. (1931). Enzootic Hepatitis or Rift Valley Fever. *Jl. Path. Bact.*, xxxiv.

Fotheringham, W. (1933). Annual Report of the Chief Veterinary Research Officer for the year 1933. Dept. of Agric., Colony and Protectorate of Kenya. Published 1934.

——. (1934). Conference of Governors of British East African Territories. Conference on Co-ordination of Veterinary Research, Kabete, January, 1934.

——. (1934). Annual Report of the Chief Veterinary Research Officer for the year 1934. Dept. of Agric., Colony and Protectorate of Kenya. In Press.

——. (1936). Conference of Governors of British East African Territories. Conference on Co-ordination of Veterinary Research, Kabete, January, 1936. In Press.

Klotz, O., and Belt, T. H. (1930). *Amer. Jl. Path.*, vi, pp. 663-668.

Kuhn, P. Ergebnisse von Untersuchungen der sudafrikanischen Pferdesterbe. *Cent. Bakt., Abt.* 1, Ref., 1911, 1, Beil., 31. Quoted by Cowdry, E. V., in Filterable Viruses. Rivers, T. M. (1928).

Nieschulz, O. (1932). Over de infectie van muizen met het virus der Zuid Afrikaansche Paardeziekte. *Tijdschs. v. Diergeneesk.*, lix, No. 24, pp. 1433-1445.

NOTES ON MALARIA AND TETANUS IN MONKEYS.

By W. C. Osman Hill, M.D., Ch.B., C.M.Z.S.,
Professor of Anatomy, Medical College, Colombo.

I. Curious Effect of Malaria on a Monkey.

Malaria in monkeys is probably of more importance than has hitherto been realised. Wenyon (1908) describes a number of parasites akin to human malaria from various primates. It is remarkable that the South American monkeys appear to be frequently infested with intracorpuscular protozoa. In this connection my experience with a squirrel monkey (*Saimiri sciurea*) which suffered from a malarial fever and recovered is of some interest.

In November 1934 I purchased a pair of the above monkeys in Genoa and brought them to Colombo where they have resided ever since. They both appeared healthy at the time of purchase, the male being the more active and stronger of the two. After about a week, however, he seemed less aggressive than before, and, though eating his food, he did not seem so anxious for it as at first. He did not get any worse before arriving in Colombo, but after about a week there he became definitely unwell and off his food. He could only be persuaded to eat with difficulty, and after some days insects were the only things that attracted his attention. He got thinner and appeared anæmic. The female remained healthy throughout.

I consulted Mr. M. Crawford, Government Veterinary Officer, and we took a blood smear. The blood was pale and examination revealed the presence of a malarial parasite in large numbers. The parasite resembled the figures given of *Plasmodium brasilianum* more than anything, so it was presumed that this had been present in the blood since the animal left his home in South America.

The patient was treated with ordinary quinine hydrochloride tablets, half a tablet being powdered and administered every other day for a little over a week. He hated the medicine and it frequently made him vomit, but he gradually improved on his diet of grasshoppers, and was also persuaded to eat finely-chopped raw liver. This reduced the anæmia and thus restored him to normal strength.

At this stage his hair started to fall out, leaving him completely bald in certain places. The parts worst affected were the terminal two-thirds of the tail and the extensor aspect of the thighs and knees; but hair also fell from most other places to some extent. The scalp was the least affected. As recovery progressed the hair grew again, but was of different character from the normal. At the same time the hair on the less affected parts was moulted and

replaced by hair of abnormal character. The new hair was in all places coarse and lacking in pigment, and was less thickly planted than it should be. The terminal black portion of the tail was replaced by black hair, but the individual hairs were shorter than the normal and sparsely planted. Elsewhere the new hair lacked the greenish hue of the normal monkey, being almost white and longer than usual, giving a shaggy unkempt effect on the body. The normally orange parts of the forearms and tibial regions seemed little affected, whilst the central portion of the scalp remained dark.

This condition remained for a little over a year, and I had given up hopes of improvement in appearance, although his general health was now good and his appetite up to normal. But in April 1936 the dark patch on the crown seemed to be increasing in area and shortly afterwards the whitish hairs on the back were falling out and being replaced by normally coloured dark hairs again. At the time of writing the improvement is very marked, but patchy, for the normal has not been attained all over. The terminal black tip to the tail is not yet as thick and bushy as in the female, but I have no doubt the improvement will continue and eventually bring him back to normal in every respect.

The interest of this case lies in its relation to the well-known fact of malaria being one of the most important ætiological factors in premature greying of the hair in man, and especially of those rare types of very rapid canities.

Reference.

Wenyon, C. M., (1908). *Protozoology*, II. p. 972, London.

II. Tetanus in Monkeys.

As I have now had experience with no less than six monkeys affected with tetanus (one of them on two occasions), I append below some observations on the progress of the disease and the effects of treatment, hoping they will be of use to physicians and veterinarians who are faced with similar circumstances.

Ætiology.

All the monkeys have been macaques of the subgenus *Zati*, one a South Indian Bonnet Macaque (*Z. radiata*) and the others Toques (*Z. sinica*).

The South Indian specimen was a male, and was the individual who developed the disease on two separate occasions. The Toques were females in two instances and males in the other three.

As regards age most were adults or subadults, but one of the male Toques was a young animal about a year old. One female was menstruating at the time of the onset of the disease.

The most important predisposing factor was the presence of a surface wound. This was present in every case. The site of the

wound varied, being most commonly an abrasion around the waist due to the wearing of too tight a belt. In the other cases it was a wound on some other part of the body—a bite from another monkey. Whatever the situation the wound was in all cases a suppurating one with thickened edges and deep pockets within, and thus forming an admirable site for growth of the *Bacillus tetani*. The organism is probably picked up from the soil, which in most Colombo bungalows seems to be infected with it. Whenever a monkey gets a wound it proceeds to stop the bleeding by rubbing it in the soil, thus introducing the organism.

Symptomatology.

The primary wound may be so small as to escape notice, so that it is impossible to say what the exact incubation period is. The first symptoms of tetanus may be the first indication of anything wrong with the animal. But in those cases where a wound has been discovered and left untreated the tetanic symptoms have been exhibited in about a week's time.

The first signs that the animal is developing tetanus are (i) a diminution of activity and (ii) a peculiar slowness in eating food—a great contrast to the macaque's usual ravenous appetite. The animal moves about but little and the movements are laboured and stiff. The diminution in activity progresses rapidly, affecting further groups of muscles, the most obvious being the masticatory muscles. The animal is only able to open its mouth a short distance, but for a time it is still able to carry on " lip-smacking." Eventually even this is prevented by the spasm of the orbicularis oris and other lip muscles, and the face takes on the typical *risus sardonicus* from the spasm of the risorius and platysma, the latter standing out as a firm band in the neck. Meantime the trunk and limb muscles have also gone into a spastic condition. Opisthotonos is produced, with the occiput touching the shoulders. The limbs are flexed or semi-flexed at the elbows and knees, the hips are extended and the hands, fingers, feet and toes are firmly flexed, and can only be partially extended by passive movement between the spasms. The animal is acutely suffering at this time, though not in any pain. Sores may develop on parts exposed to pressure or friction unless the animal is continually changed from side to side. There is no loss of consciousness and the poor creature recognises its owner until the fatal issue supervenes. Eye movements are also possible till the close of the disease. Respirations appear to be increased in frequency, the thorax being relatively immobile and the diaphragm chiefly responsible for the respiratory movements.

Prognosis.

The disease, I think, would always be fatal if left untreated, but the fatal issue would appear to occur at a variable time from

onset according to the severity of the infection, which, in turn, depends upon the size and character of the original wound. One large male toque had a very severe, almost a complete annular wound round his waist, and he died in spite of treatment; whereas another with a less severe primary wound recovered although he remained untreated until two days after he had been in full spasm.

The immunity conferred by serum treatment is of short duration. The animal that developed the disease a second time did so exactly twelve months after his first attack.

If adequately treated from an early stage of this disease with the method advocated below, and provided the animal is well nourished and nourishing food is administered during the illness, I consider that all cases are curable.

Treatment.

The only treatment that promises any chance of success is the intrathecal injection of large doses of antitoxin. This should be done from the time the disease is recognised, and, although some of my cases recovered when the injection was not commenced till the body was in full spasm, naturally I do not recommend waiting for this to happen.

The injection is given with a serum needle through one of the intervertebral intervals above the line joining the two posterior iliac spines. The needle passes easily through the superficial muscles, then some resistance is felt due to the ligamentum subflavum. Once through this the injection is made. I give a full phial (3,000 British units) of antitoxin every other day in average cases and continue the injections until the tonus of the limb muscles is approaching normal. Usually three or at most four injections are required for an average-sized monkey. I give double doses where the infection, as judged by the state of the primary wound, appears to warrant it.

It is most essential to pay attention to the state of the primary wound and to clean it up. This requires great patience, as every little pocket needs disinfecting, otherwise constant reinfection of the nervous system *via* the nerve trunks is going on, and the antitoxin is being constantly neutralised thereby. The animal does not in my experience object to the process, nor to the injection. It always realises that something is being done to help it, and after the operation tries to smack its lips in thanks.

Care must be taken to prevent pressure sores. These rapidly develop over bony points if the animal is left lying in one position for too long. A good soft bed underneath and fairly frequent changes of position from one side to the other are recommended.

As regards general nursing, it is best to keep the patient in a darkened place and to remove all sources of stimulation likely to induce the spasms. Feeding is a difficult matter, and when trismus is present hand feeding with drops of fluid is all that can

be expected. The animal automatically tries to eat solid food by hasty snatches with its teeth the moment it is sufficiently recovered to do so.

Recovery.

This is the most dramatic and interesting feature of tetanus in monkeys. If treatment is beneficial, its effects are soon felt, and once on the way to recovery progress is rapid provided care is taken of the original wound.

Relaxation of the jaws is one of the first signs of recovery. They do not relax completely, but only slightly to begin with. The opisthotonos then improves and the animal proceeds to turn its head about and looks for its caretaker's approach. In four or five days the lower limbs recover and when these are sufficiently flexible the animal tries to use them and soon learns to raise itself up by getting its back against a wall or box, and, having done so, balances itself on its legs. After a day or two it hops about in this position like a kangaroo, supporting itself by its stiffened tail. It will take its own food at this stage if the plate is placed on a box level with its head. The arms at this stage are held stiffly with the hands firmly flexed and held against the groin. The abdominal muscles are also still in spasm at this stage. About two days later recovery begins in the arms, though some stiffness remains both in them and the abdomen for two or three more days. When the arms are sufficiently recovered the animal uses them in feeding, and once this has occurred further treatment is unnecessary.

I conclude with the following analysis of the cases I have seen. They are given in chronological order.

TABLE I.

Analysis of Seven Cases of Tetanus in Monkeys.

Species.	*Sex.*	*Age.*	*Wound.*	*Treatment.*	*Result and Remarks.*
1. *Z. sinica*	♀	Post-pubertal	Waist	Nil.	Died (Disease not recognised till post-mortem)
2. *Z. radiata*	♂	Subadult	Waist	As advocated above	Recovered
3. *Z. radiata*	♂	Same animal as (2) one year later	Finger	Same	Recovered
4. *Z. sinica*	♀	Pre-pubertal	Finger	Same	Recovered
5. *Z. sinica*	♂	One year	Waist	Same	Consulted too late; died
6. *Z. sinica*	♂	Adult	Waist	Same	Died
7. *Z. sinica*	♂	Adult	Thigh	Nil.	Killed

FOWL PLAGUE AND NEWCASTLE DISEASE.

By Professor Dr. R. MANNINGER,
Budapest, Hungary.

SEVERAL years ago I published a report[1] on investigations which, along the line of a study on the plurality of filter-passing viruses, were carried out in order to clear up the immunobiological features of the causative agents of the classical fowl plague and the disease described by Doyle[2] and Picard[3] under the name Newcastle disease of fowls, or pseudo-fowl plague. During these investigations I succeeded by means of an experimental arrangement in modifying the virulence of the Newcastle virus, with the result that the disease caused by the Newcastle virus clinically and anatomically exhibited no difference when compared with cases of the classical fowl plague. Moreover, the disease, known to have a fairly long incubation and a relatively prolonged course, changed its character so that, following a short incubation period, it ran a rapid course. Further, I was able to demonstrate that the immunity conferred upon a number of fowls through treatment, partly with the modified virus and partly with the virus of the classical fowl plague, proved equally satisfactory against both infective agents under strict observance of appropriate quantitative conditions.

Doyle, in a highly interesting article,[4] disagrees with the conclusions drawn from my previous experiments quoted above, and a little earlier they were also discussed in an article by Picard.[5] Doyle's most important objections may be summarised as follows: (1) He contends that I omitted to examine the susceptibility of pigeons to the Newcastle virus. Experiments in this direction would have convinced me that pigeons, while showing a great resistance against the virus of fowl plague, are remarkably susceptible to the Newcastle virus. (2) While studying the immunological properties of the viruses I found it satisfactory—according to Doyle—to examine immunised animals only, instead of completing my investigations with the examination of immunising sera by means of the so-called neutralisation test. (3) It is claimed that an attempt of his to repeat my experiments on the increase of virulence did not confirm my experimental results, according to which the infectivity of the blood plasma may be exalted through animal passages.

The above critical remarks of Dr. Doyle induced me to carry out a new series of experiments the results of which are presented as follows:—

(1) Owing to the well-known fact that pigeons manifest a varying susceptibility to the infective agent of the classical fowl plague, I purposely omitted to deal in my previous article with the effect of the viruses of fowl plague and Newcastle disease on

pigeons. Nevertheless I did not omit to examine the effect of the Newcastle virus on pigeons at the time when, by the courtesy of Dr. Doyle, I received a sample of that virus, and, in harmony with Doyle's experience, I too have found that pigeons may be susceptible to the morbid effect of this virus. In December of 1930, as well as in January and February of 1931, we infected 13 pigeons by inoculating the infective material from bird to bird. Twelve of the inoculated birds contracted the disease and, after showing convulsions and paralytic symptoms, died within four to 13 days after the infection, while with the inoculation of the thirteenth animal the series became interrupted.

In November of 1935, *i.e.*, after the publication of Doyle's second article, we again examined the virus, which meanwhile was kept alive in fowls, and tried to determine its effect on pigeons, with the result that of twelve birds receiving the lethal dose for fowls in a 10,000 to 100,000 times stronger quantity none contracted the disease. It may be concluded from this experiment that the effect of the Newcastle virus on pigeons is by no means a constant one, and consequently the pigeon test, contrary to Doyle's opinion, cannot be looked upon as determinative for the differentiation between the virus of fowl plague and Newcastle disease.

(2) Though the examination of the behaviour of actively immunised animals against the infective agents, with due consideration of the quantitative conditions, in my opinion proves at least as reliable a method for the establishment of their immunological peculiarities as the neutralisation test, yet having regard to Doyle's argument we found it worth while to immunise to a high degree one goose with the virus of the classical fowl plague and another one with the Newcastle virus. One c.c. of blood serum of each animal was then mixed in a test tube with a definite number of lethal doses of the virus of fowl plague and Newcastle disease respectively, and the mixture after two hours was inoculated into susceptible fowls. The results of these experiments are shown in Tables I and II. From these it can be seen that the two blood sera neutralised uniformly both infective agents, thus suggesting once more that from an immunobiological point of view the two agents exhibit no difference.

However, considering the diverse results of immunological tests obtained by various authors, it might be admitted that the virus of fowl plague may under varying conditions present different types or strains. If this is the case then we must assume that the latter may change the one into the other.

(3) With reference to Doyle's statement that he did not succeed in an attempt to exalt the Newcastle virus in the blood plasma and thus to produce the clinical or pathological picture of the classical fowl plague, I wish to remark that his experiments were not carried out on exactly the same line as my experiments. In my experiments I succeeded in increasing the concentration of the virus and

TABLE I.

Number of Fowl.	*Immunising Serum Against—*	*Virus.*	*Number of Lethal Doses.*	*Result.*
308	Fowl plague	Fowl plague	100,000	Died within 4 days
309	„	„	100,000	„ 3 „
310	„	„	10,000	„ 4 „
311	„	„	10,000	„ 7 „
312	„	„	1,000	„ 5 „
313	„	„	1,000	„ 5 „
314	„	„	2	Remained healthy
315	„	„	2	„
316	„	„	1	„
317	„	„	1	„
318	„	Newcastle	100,000	Died within 6 days
319	„	„	100,000	„ 5 „
320	„	„	10,000	„ 7 „
321	„	„	10,000	„ 5 „
322	„	„	1,000	Remained healthy
323	„	„	1,000	Died within 7 days
324	„	„	2	Remained healthy
325	„	„	2	„
326	„	„	1	„
327	„	„	1	„
328	—	Fowl plague	2	Died within 36 hours
329	—	„	1	„ 36 „
330	—	Newcastle	2	Died within 36 hours
331	—	„	1	„ 48 „

TABLE II.

Number of Fowl.	*Immunising Serum Against—*	*Virus.*	*Number of Lethal Doses.*	*Result.*
332	Newcastle	Fowl plague	100,000	Died within 3 days
333	„	„	100,000	„ 2 „
334	„	„	10,000	„ 5 „
335	„	„	10,000	„ 5 „
336	„	„	1,000	„ 4 „
337	„	„	1,000	„ 7 „
338	„	„	2	Remained healthy
339	„	„	2	„
340	„	„	1	„
341	„	„	1	„
342	„	Newcastle	100,000	Died within 7 days
343	„	„	100,000	„ 7 „
344	„	„	10,000	„ 5 „
345	„	„	10,000	„ 8 „
346	„	„	1,000	„ 6 „
347	„	„	1,000	„ 5 „
348	„	„	2	Remained healthy
349	„	„	2	„
350	„	„	1	„
351	„	„	1	„

consequently the virulence of the blood plasma by inoculating the blood without delay at a very early stage of the disease, *i.e.*, at the onset of the fever, from bird to bird. Such a procedure is to be followed owing to the circumstance that with the progress of the disease the virus gradually disappears from the blood, remaining in a less potent form only in some organs. Doyle, however, meeting some difficulties in the exact determination of the onset of the fever in his experimental birds, transmitted the infective material from bird to bird in a different way, *viz.*, arbitrarily always during the seventy-second hour after the artificial infection. This experimental divergence explains satisfactorily the difference existing between the results of his and my experiments.

As an interesting fact it may be mentioned that since the publication of my previous article several papers have appeared containing data which also seem to demonstrate that from an ætiological point of view Newcastle disease is identical with the classical fowl plague.

Apart from the fact that there may be rare cases of classical fowl plague showing much the same protracted course that is characteristic of Newcastle disease, Nakamura, Oyama and Tomonaga[6] were successful in modifying the virus of the disease occurring in Japan, which in Doyle's opinion is identical with the Newcastle disease, by repeated passage through birds, just as happened in my experiments with the original Newcastle virus. This virus, according to a recent publication by Picard,[7] may change its character even spontaneously, so as to be present in considerable amount in the blood of the infected birds. It is further stated by the authors named that the disease produced by the modified virus may, in accordance with my statements, manifest itself by much the same clinical symptoms as may be observed in cases of classical fowl plague, and, according to Fukushima,[8] no difference exists even with regard to *post-mortem* lesions and histological changes.

At present there seems to be no doubt that the particular susceptibility of pigeons to the Newcastle virus pointed out by Doyle, in the light of the statements of other workers provides no sound basis for the differentiation of the virus of classical fowl plague and Newcastle disease. Rachad,[9] experimenting with Doyle's original material and de Blieck,[10] with that obtained from Picard, could not infect pigeons. These records appear to corroborate my own results showing that the Newcastle virus at one time may, and at another may not, induce the disease in pigeons.

The above-mentioned statements appear to demonstrate uniformly that Newcastle disease and the classical fowl plague represent an ætiological unity, though it must be admitted that in

its clinical manifestations, particularly in its protracted course, Newcastle disease doubtless differs from fowl plague.

At all events, the causative agent of fowl plague and of the Newcastle disease respectively seems to be a virus distinguished by a considerable lability.

References.

[1]Manninger. (1932). *Arch. wiss. prakt. Tierhlk.*, lxv, p. 256.
[2]Doyle. (1927). *J. Comp. Path. & Therap.*, xl, p. 144.
[3]Picard. (1927). Dept. Agr. Dutch East India, *Vet. Bull.*, 65.
[4]Doyle. (1935). *J. Comp. Path. & Therap.*, xlviii, p. 1.
[5]Picard. (1934). *Zentralbl. Bakt. I. Orig.*, cxxxii, 1, 440.
[6]Nakamura, Oyama and Tomonaga. (1932). *J. Jap. Soc. Vet. Sci.*, xi, p. 298.
[7]Picard. *Loc. cit.*
[8]Fukushima. (1933). *J. Jap. Soc. Vet. Sci.*, xii, p. 147.
[9]Rachad. (1934). Dept. Agr. Egypt, *Vet. Bull.*, 140.
[10]See : Picard. (1934). *Zentralbl. Bakt. I. Orig.*, cxxxii, p. 440.

CONTENTS

GENERAL ARTICLES.

PAGE

A Study on Immunity Produced by Infection with Attenuated Culture-Strains of *Histomonas meleagridis*. By Ernest Edward Tyzzer, M.D. 285

Toleration of Naganol (Bayer 205) by Horses. By S. C. J. Bennett, D.SC., M.R.C.V.S. 304

Primary Irido-Cyclitis in Fowls ; a Condition Distinct from the Eye Lesions Occurring in Neuro-lymphomatosis. By H. P. Bayon 310

The Isolation of Brucella abortus from the Milk of Cows with Negative Blood Reactions to the Agglutination Test. By T. M. Doyle 320

Paralysis in Fowls and Scrapie in Sheep. (A Comparison). By A. Brownlee, B.SC., M.R.C.V.S. 328

Results of Passage of Human and Monkey Strains of *Brucella melitensis* through Pregnant Heifers. By D. E. Wilson (Medical Officer) and S. A. Evans (Veterinary Research Officer, Tanganyika Territory) 336

Experiments on Immunisation against *Pasteurella septica* Infection. By F. W. Priestley 340

A Note on the Association of the Virulence of *Pasteurella septica* with Capsulation. By F. W. Priestley 348

The Value of Vaccination with Live Cultures of *Br. abortus* for the Prevention of Abortion. By H. E. Keylock, F.R.C.V.S. 350

Thrombo-Angiitis obliterans. By E. D. Telford, F.R.C.S. 361

REVIEWS.

Handbook for Veterinary Surgeons. By Fred Bullock, LL.D. ... 363

Bernhard Bang. Selected Works. Edited by Vald. Adsersen ... 363

NOTICE TO SUBSCRIBERS.

Communications, exchange journals, specimens, etc., for the Editor should be addressed to Sir John M'Fadyean, Highlands House Leatherhead, Surrey.

BINDING CASES.—Maroon Full Bound Cloth. Can be obtained from the Publishers and Agents, Price 3s. 6d., post free.

PARÔPHYROXIÂ

(HEWLETT'S)

It possesses three distinctive therapeutic powers—*i.e.*, Antiseptic, Purgative and Carminative—and is indicated in all cases of Colitis caused by undigested food, or irritative affections of the alimentary canal, partly no doubt by removing acrid irritating secretions or foreign materials, neutralising gaseous fluids, depleting the congested blood vessels, and arresting the distended and tympanitic condition of the colon, rendering it the safest and most satisfactory purgative in these cases. It is peculiarly adapted for those cases of Colic so frequently met with in aged horses, who are generally described as " Gross Feeders," where the food is bolted and not sufficiently masticated owing to the teeth being faulty, and where the digestive organs are impaired from a loss of tone in the system, the circulation bad, and the necessary amount of gastric juice is insufficient to digest the food. It is very extensively used in Scotland by numerous practitioners for the Colic caused by coarse feeding which produces impaction with painful contraction of the muscular coat of the bowels accompanied with tympanites.

It is in these cases that Parôphyroxiâ has a very marked effect in reducing the spasm, arresting fermentation, and subduing the drum-like condition of the colon. The large amount of clinical evidence adduced in its favour by various experienced and sound practitioners commends it so strongly that it is now placed at the head of the list of the different agents employed in the general treatment of Colic.

It should be well borne in mind that as the drug is purgative in its action no physic ball or other purgatives should be administered, but if the practitioner deemed it necessary to follow up the treatment with another draught, some simple antispasmodic would not be contra-indicated.

Price 4s. 6d. per lb. W. Quarts 4s. 3d. per lb.

Agents for the Vaccines and Sera of the Bacteriological Institute, Tours.

Tuberculin (Subcutaneous, Intradermal and Ophthalmic), Mallein, Vaccines for Canine Distemper (Prophylactic and Curative), Pneumonia, Strangles in Horses and Mastitis, etc. Pyovaccine for Sores and Contagious Lymphangitis.

Also Agents for the Jensen-Salsbery Biological Products, Pasteur Institute, Paris, etc.

Only Supplied to Members of the Veterinary Profession.

C. J. HEWLETT & SON, LTD.,

Wholesale Druggists and Manufacturing Chemists,

35-42, CHARLOTTE STREET, & 83-85, CURTAIN ROAD, LONDON, E.C.2.